

Molecular Aspects of Hammer Head Ribozyme Isolated From Virus & Its Influence on RNA Biology

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Abstract

The field of RNA biology is now advancing sporadically. Hammer head ribozyme motif has a great role to play in RNA biology. This was first isolated from viruses. They are used to cleave other RNA molecules; can be vastly used in genetic manipulations. This paper gathers the information regarding the functions, unique properties of Hammer head ribozymes.

Keywords: - Virus, hammer head ribozymes, RNA biology, Functions

INTRODUCTION

The discovery of the hammerhead ribozyme

The hammerhead ribozyme catalytic motif was discovered erstwhile by comparing the sequences of certain plant viroids and virusoids that underwent site-specific, self-catalysed cleavage as part of their replication process. The motif was found to encompass three base-paired helices (I-111), connected by two single-stranded regions and a bulged nucleotide. The single-stranded regions were shown to be invariant, except at position 7, and were presumed to form a conserved catalytic

core. In contrast, the base-paired helices (1-111) showed quite little sequence similarity, with the exception of Y11.1, terminating stem 11, and A15.1-U16.1, terminating stem I11. In nature, two of the hammerhead's helical arms are terminated by nucleotide loops and thus the ribozyme catalyses the intramolecular (in-cis) cleavage of a phosphodiester bond to yield a 2', 3'-cyclic phosphate and a 5'-hydroxyl product. Intermolecular (in-trans)-cleaving ribozymes can be created synthetically by removing a loop terminating one of the helical arms (De la Pena et al., 2001). Dividing the ribozyme

in this manner creates a cleavable substrate strand, which can be typically bound by the enzyme strand. In this case, the enzyme strand is able to bind and cleave many of substrate strands and thus perform catalysis in a similar manner to protein enzyme. There are three possible constructs of this type, which have been termed I/II, I/III and 1411, depending on which helices are available to bind the substrate strand, all of which are catalytically active. In the I/III construct, where anucleotide loop terminates stem TI, the enzyme strand contains almost all of the conserved nucleotides; the remaining conserved nucleotides are located in the substrate strand and form part of a cleavage triplet. The ease of chemical production of this type of construct, coupled with it being the most suitable for gene inhibition, has resulted in it typically being the most frequently studied of the three constructs (Saksmerprom et al., 2004).

RNA catalysis in biology is mainly considered as an unusual feature of the RNA molecule. The first demonstration and analysis that polypeptides can be biological catalysts was published as early as 1927, and consequently, the discovery in the 80s of catalytic RNAs or ribozymes was a really late paradigm change. In

1983, the self-splicing Group I intron was reported as the first discovered catalytic RNA (Benseler et al., 1993). It was delineated in the ciliate protozoa *Tetrahymena thermophila*, although similar introns can be seen in some prokaryotic genomes and the mitochondria and chloroplast DNA of diverse eukaryotes. The second example of a predominant ribozyme to be discovered was the RNase P involved in tRNA maturation, which had a key biological role and a ubiquitous occurrence (Bertrand et al., 1994). Quite intriguingly, and despite the bacterial origin of the chloroplast and mitochondria, the RNase P in these organelles is a protein that functions without RNA. The third reported catalytic RNA was a tiny ribozyme (~55nt), the self-cleaving hammerhead ribozyme (HHR), which was found in a group of atypical plant pathogens with the small circular RNA (circRNA) genomes such as viral satellite RNAs and viroids. Since then, a few more examples of the either natural or artificial ribozymes have been discovered, including the ribosome, a singular catalytic RNA that catalyzes the peptide bond formation, the central chemical reaction in extant biology (Bertrand et al., 1996). This landscape strongly supports the hypothesis of the prebiotic RNA world, where the first self-replicating organisms were based on

RNA as both the genetic material and act as catalyst (Bertrand et al., 1994). Whereas modern proteins would have replaced most of these ancient catalytic RNAs, some of them have remained in current organisms performing different functions. Among all the known ribozymes, there is the enigmatic family of small (<200 nt) self-cleaving RNAs, which catalyze a simple intramolecular transesterification in a highly sequence-specific manner (Eckstein et al., 1997).

Although there is quite a little apparent sequence variation, the contact appears to be present in most if not all hammerhead sequences (Chatterjee et al., 1992). Although the minimal hammerhead has a specific turnover rate of approximately 1 min⁻¹, full-length sequences that include a tertiary contact, described in Section 3, are up to 1000-fold more active (Cech et al., 1990).

ENZYMOLGY

Rate enhancement—The rate of non-site-specific, spontaneous decay of RNA is quite highly dependent upon the secondary structural context, but is on average about 10⁶ min⁻¹. Hence, the rate enhancement provided by an optimized minimal hammerhead is on the order of 10⁷, and for the full-length natural

hammerhead can be as much as 10⁹ (Burgin et al., 1996). To achieve this magnitude of rate enhancement, not to mention site specificity, the hammerhead ribozyme must adopt multiple effective catalytic strategies simultaneously. Each of these is separated perhaps somewhat artificially and analyzed below (Chowrira et al., 1994). Metal ions and catalysis—originally, it was believed that all the ribozymes, including the hammerhead ribozyme, were obligate metallo enzymes. Mg²⁺ ion is assumed to be the biologically relevant divalent cation, although the hammerhead is active in the presence of a variety of divalent cations (Christoffersen et al., 1994). Proposed roles for the Mg²⁺ ion in catalysis included both acid and base catalysis components (with Brønsted and Lewis variants of this proposal articulated) as well as a direct coordination of the pro-R non bridging phosphate oxygen of the scissile phosphate for transition-state stabilization. Mg²⁺ ion has also been specifically implicated in structural roles that facilitate formation of the active ribozyme (Clouet-D'Orval et al., 1996).

In 1999, it was demonstrated that the hammerhead, along with the hairpin and VS ribozymes (but not the HDV ribozyme) could also function in the

absence of the divalent metal ions as long as a high enough concentration of positive charge was present (molar quantities of Li^+ , Na^+ , or even the nonmetallic NH_4^+ ion permit cleavage to take place). The researchers suggested that ribozymes were not strictly metallo enzymes (Cotton et al., 1989). Considering the volume of research devoted to understanding the mechanistic roles of divalent metal ions in hammerhead ribozyme catalysis and the fundamental and basic tenet of ribozyme enzymology that all ribozymes are metallo enzymes, it was unexpected to find that at least three of the four small naturally occurring ribozymes can function much reasonably efficiently in the absence of divalent metal ions; a fact that was much discovered in the course of performing experimental controls for time-resolved crystallographic freeze Trapping experiments in crystals of the minimal hammerhead ribozyme (Craig et al., 1979). It majorly now appears that RNA folding and nonspecific electrostatic transition state stabilization accounts for much, if not all, of the catalytic enhancement over background rates found with these ribozymes (Crisell et al., 1993).

For example, hammerhead, which is much considered to be an optimized hammer head ribozyme sequence for single-

turnover reactions, cleaves only threefold faster in the presence of 10-12mM MgCl_2 and 2 M Li_2SO_4 than it does in the presence of 2 M Li_2SO_4 alone (Dahm et al., 1991). The rates of hairpin and VS ribozymes in 2 M Li_2SO_4 actually exceed those that measured under —standard low ionic strength conditions, and the rate of cleavage for then on-optimized hammerhead sequence used for crystallization is fivefold enhanced in 3 M Li_2SO_4 . The non-optimized sequence availed for crystallization tends to form alternative, inactive structures in solution, such as a dimer of the enzyme strands, which dominate at lower ionic strength (Dahm, et al., 1993). This result implied that any chemical role of the Mg^{2+} ion in the ribozyme reaction was likely to be one of comparatively nonspecific electrostatic stabilization rather than more direct participation in the chemical step of catalysis (de-Feyter et al., 1996). Furthermore, the result implied that the RNA itself was an active participant in the chemistry of catalysis rather than serving as a passives caffold for binding metal ions that served the roles of general acid and base catalysts (Denmann et al., 1994). With the subsequent structural elucidation and assessment of the hairpin and full-length hammerhead structures, it was, in fact, revealed that RNA bases and other

functional groups were positioned to garner the moieties likely responsible for acid–base catalysis. Acid–base chemistry—originally, hydrated Mg^{2+} and the other hydrated divalent metal ions were thought to play the direct chemical role of general base and general acid in ribozyme catalysis, with the RNA it serving as an ancillary and typically passive scaffold upon which metal ions would bind and would be positioned in the active site. With the discovery that the hairpin, hammerhead, and VS ribozymes were not strictly metallo enzymes, it became the apparent that in at least these three cases, the RNA itself must be an active participant in the chemistry of catalysis rather than serving merely as a metal ion-binding scaffold. The crystal structure of the hairpin ribozyme, is in contrast to the HDV ribozyme that is in fact a metallo enzyme, soon validated this prediction. It was not apparent from the crystal structure of the minimal hammerhead, Are the functional groups might be involved in acid–base catalysis. Consequently, the focus and aim of biochemical mechanistic investigations in the hammerhead turned to this problem. The invariant core residues G12 and G8 in the hammerhead ribozyme were quiet finally identified in 2005 as likely candidates for participation in acid–base

chemistry by careful purine modification studies and researches conducted by John Burke and coworkers.^{45,46} Substitution of G12 (pKa9.5) with inosine (pKa 8.7), 2,6-diaminopurine (pKa 5.1) or 2-aminopurine (pKa 3.8) shifts the reaction rate profile in a manner consistent with G12's suggested a key role in general base (or acid) catalysis without significantly perturbing ribozyme folding. Similar substitutions at G8 also implicated this invariant residue in acid–base catalysis, but in this case, the modifications also partially inhibited ribozyme folding. These experiments could not mainly determine specifically whether an individual nucleotide, such as G12, was the general acid or the general base, but clearly implicated G12 and G8 in acid–base catalysis (Hao et al., 2006).

KINETICS—the Minimal Hammerhead Ribozyme, Under “Standard” Reaction Conditions

There are many uses for hammerhead ribozymes (hhRz), as they are quiet short catalytic RNA molecules which anneal to and cleave other RNA molecules—typically transcripts of genes—leading to the knockdown of gene expression (Carlyle et al., 2004). There are many other means of gene suppression via transcript cleavage, and they include the widely availed RNAi (1). RNAi is

effective at low concentrations can be delivered via multiple pathways, allow for tissue specific expression and are generally accepted to be very highly specific and non-toxic (2). Nevertheless, ribozymes (3), including the hhRz (4), have relatively simple catalytic domains and can be used to target specifically RNA. They can potentially discriminate between the single-base polymorphisms, target introns and sub-cellular compartments, do not require helper proteins to function and hence, can be tested in vitro. They are also easily modifiable and also have been modified in ways that allow for more efficient (Wong et al., 2009), more specific as well as allosteric applications involving RNA, and small molecules or protein triggers. Finally, ribozymes can work in organisms that are devoid of the typical RNAi mechanism found in eukaryotes, such as bacteria or even in simple solutions in vitro. For these reasons, hhRz are observed as general and malleable means of transcript modification and gene therapy, with applications in biosensors and synthetic biology as well. The hhRz is one of the multiple structural motifs of ribozymes that can cause both cis- and trans-cleavage of RNA molecules with certain properties. The model of the hhRz, comprises of three largely independent parts: stem I, stem II (including the

catalytic core) and stem III. Atrans cleaving ribozyme annealed to the target substrate strand. Stems I and III are chosen to be complementary to the substrate strand, which must include an NUH triplet. Stem II is found next to the catalytic cores conserved GAAA and CUGANGA. Generally assessing, a designer would pick an empirically proven stem II and catalytic core, then vary the two 'arms' (call them I and III) so they would form stems I and III, with pre-specified characteristics, with the targeted substrate (Chen et al., 2009).

CONCLUSION

Hammer head RNA ribozymes have a great role to play, because of its catalytic property, and has the ability to cleave other RNA regions. This characteristic feature can be utilized in genetic engineering in upcoming future. The major properties of Hammer head ribozymes are discussed in this paper.

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