CATALYTIC STRATEGIES OF NUCLEOLYTIC RIBOZYMES

DARRIN M. YORK

Center for Integrative Proteomics Research, Laboratory for Biomolecular Simulation Research and Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ 08854, USA

My view of the present state of research on catalysis by ribozymes in molecular machines

The ability of RNA molecules to selectively and efficiently catalyze complex chemical transformation has vast implications. Our understanding of the mechanisms of RNA catalysis have been greatly advanced by the study of small nucleolytic RNA enzymes, or ribozymes, that have evolved naturally in viruses and living organisms, or artificially through high-throughput *in vitro* selection techniques [1]. Experimental structural and mechanistic work, along with computational simulations have provided deep insight into the mechanism of these model ribozyme systems. Very recently, there has been a surge in progress in the determination of crystallographic structures of ribozymes [2–6] that have provided a departure point for theoretical investigations that aim to bridge the gap between structural and mechanistic measurements, and provide a detailed dynamical picture of mechanism at atomic-level resolution. In this way, molecular simulations have the potential to unify the interpretations of a broad range of experimental data and establish a consensus view of mechanism [7]. Ultimately, multiscale simulations, together with experiments, afford the tools needed to gain predictive insight into catalysis, including control factors that regulate selectivity and reactivity, that may guide rational design efforts.

In the present work, results from multiscale molecular simulations are presented for a series of ribozymes for which crystallographic data has recently become available, including the twister [2], Varkud satallite virus (VS) [3] and pistol [4] ribozymes. These results uncover recurring themes, as well as new twists, in the catalytic strategies taken by ribozymes that are apparent only when broadly analyzing their structure, biochemical characterization and detailed mechanisms predicted by molecular simulations. The interpretations of experimental data afforded by new multiscale molecular simulation results uncover general principles and provide predictive insight into the catalytic mechanisms of nucleolytic ribozymes that may guide rational design efforts.

My recent research contributions to catalysis by ribozymes in molecular machines

Recently we have made several major advances in the development of multiscale quantum models for biocatalysis simulations that allow new insight to be obtained into the mechanisms of RNA catalysis. Briefly, these advances include the development of: (1) *ab initio* combined quantum mechanical/molecular mechanical (QM/MM) simulation methods [9] capable of treating rigorous long-range electrostatic interactions under periodic boundary conditions most commonly used to mimic aqueous solution; (2) robust methods for sampling [10] and free energy analysis [11] that allow multidimensional free energy landscapes to be calculated reliably and efficiently; (3) new models for divalent metal ions that accurately describe interactions with nucleic acids [12] in long-time molecular dynamics simulations of ribozyme systems [8, 13]. Together, these advances have made possible the mechanistic study of newly discovered ribozymes that have allowed recurring themes and new twists in the catalytic strategies of nucleolytic ribozymes to emerge [7].

As an example, recent study of the twister ribozyme [8] that has no specific divalent metal ion requirement in catalysis, and combined QM/MM simulations suggest that the active site employs a novel mode of general acid catalysis involving protonation at the N3 position of adenine. Despite this new twist, the twister ribozyme active site architecture determined by molecular simulations bears remarkable resemblance to that of the hairpin [6] and VS [3] ribozymes; two other well-studied nucleolytic ribozymes that have no explicit divalent metal ion requirements for catalysis [14]. Specifically, the active site architecture of these ribozymes has an L-platform scaffold [14] that positions a guanine residue implicated as a general base, and is locked into place by hydrogen bonding with an L-anchor (Fig. 1).

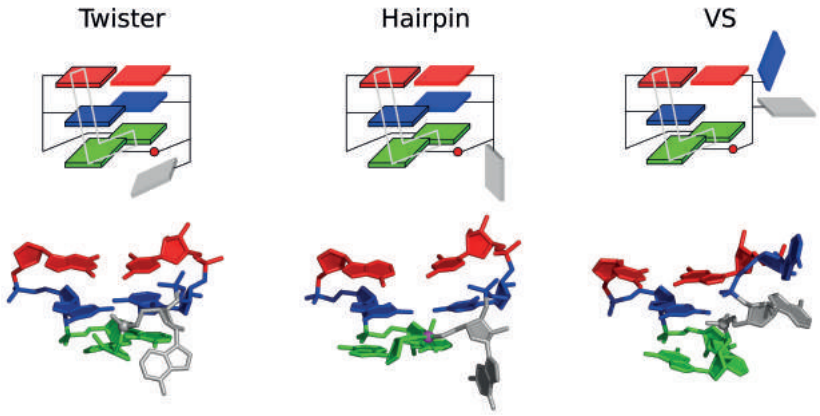

Fig. 1. Active site architecture of the twister, hairpin and VS ribozymes. (Top) Schematic showing the L-platform (indicated by an outlined letter "L") and L-anchor motifs. The scissile phosphate where cleavage occurs in shown as a red dot. (Bottom) Atomistic representation of the active site predicted from molecular simulation [8] (twister) or determined from crystallographic data (hairpin [6] and VS [3] ribozymes).

The pistol ribozyme [4], on the other hand, is a newly discovered ribozyme that has explicit divalent metal ion requirements for catalysis under near-physiological salt conditions, and has a mechanism that has stark similarities to the hammerhead ribozyme [5]. The hammerhead and pistol ribozymes share a similar L-platform scaffold as observed in the twister, hairpin and VS ribozymes, but instead of the L-anchor region that hydrogen bonds with the L-platform, these ribozymes contain a divalent metal ion binding pocket (the L-pocket) that positions a metal ion that assists in catalysis. Taken together, these results support the notion that the catalytic strategies of nucleolytic ribozymes may follow a few simple guiding principles, a detailed understanding of which may ultimately guide rational design efforts.

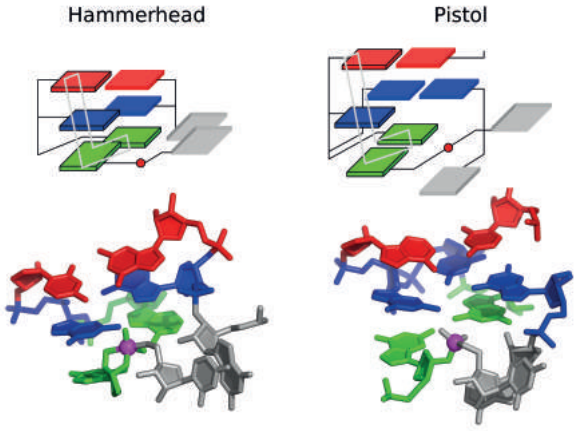

Fig. 2. Active site architecture of the hammerhead and pistol ribozymes. (Top) Schematic showing the L-platform (indicated by an outlined letter "L") and L-pocket motifs. The scissile phosphate where cleavage occurs in shown as a red dot. (Bottom) Atomistic representation of the active site predicted from molecular simulation.

Outlook to future developments of research on catalysis by ribozymes in molecular machines

The future of mechanistic research into catalytic mechanisms of ribozymes is bright indeed. The recent success of theoretical models build upon advances in experimental high-throughput methods, and structural and biochemical characterization of ribozymes. Key to pushing the boundaries of our understanding of these catalytic molecular machines is the close interplay of theory an experiment. Theoretical studies must be pressed to go beyond validation of existing experimental data to make experimentally testable predictions. At the same time, the experimental community should begin to more broadly embrace serious theoretical work and make effort to carry out new experiments motivated by results from realistic physical and chemical models. Finally, the scientific community should work to develop new ways of integrating the information gained by experiment and theory into the rational design pipeline to facilitate discovery.

Acknowledgments

The author is grateful for financial support provided by the National Institutes of Health (Grants GM62248 and GM107485). This work used the Extreme Science and Engineering Discovery Environment (XSEDE), which is supported by National Science Foundation (NSF) Grant OCI-1053575 (Project TG-MCB110101) and also in part by the Blue Waters sustained petascale computing project (NSF Grant OCI 07-25070 and Petascale Computing Resource Allocations Grant OCI-1036208).

References

[1] K. A. Harris, C. E. Lünse, S. Li, K. I. Brewer, R. R. Breaker, *RNA* **21**, 1852 (2015).
[2] Y. Liu, T. J. Wilson, S. A. McPhee, D. M. J. Lilley, *Nat. Chem. Biol.* **10**, 739 (2014).
[3] N. B. Suslov, S. DasGupta, H. Huang, J. R. Fuller, D. M. J. Lilley *et al.*, *Nat. Chem. Biol.* **11**, 840 (2015).
[4] A. Ren, N. Vušurović, J. Gebetsberger, P. Gao, M. Juen *et al.*, *Nat. Chem. Biol.* **12**, 702 (2016).
[5] A. Mir, B. L. Golden, *Biochemistry* **55**, 633 (2016).
[6] J. Salter, J. Krucinska, S. Alam, V. Grum-Tokars, J. Wedekind, *Biochemistry* **45**, 686 (2006).
[7] M. T. Panteva, T. Dissanayake, H. Chen, B. K. Radak, E. R. Kuechler *et al.*, *Methods Enzymol.* **14**, 335 (2015).
[8] C. Gaines, D. M. York, *J. Am. Chem. Soc.* **138**, 3058 (2016).
[9] T. J. Giese, D. M. York, *J. Chem. Theory Comput.* **12**, 2611 (2016).
[10] B. K. Radak, M. Romanus, T.-S. Lee, H. Chen, M. Huang *et al.*, *J. Chem. Theory Comput.* **11**, 373 (2015).
[11] T.-S. Lee, B. K. Radak, M. Huang, K.-Y. Wong, D. M. York, *J. Chem. Theory Comput.* **10**, 24 (2014).
[12] M. T. Panteva, G. M. Giambasu, D. M. York, *J. Phys. Chem. B.* **119**, 15460 (2015).
[13] T.-S. Lee, B. K. Radak, M. E. Harris, D. M. York, *ACS Catal.* **6**, 1853 (2016).
[14] T. J. Wilson, D. M. J. Lilley, *Prog. Mol. Biol. & Trans. Sci.* **120**, 93 (2013).