Deoxyribozymes to Hydrolyze DNA Phosphodiester Bonds

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Deoxyribozymes are single-stranded DNA oligonucleotides that catalyze various chemical reactions, analogous to protein enzymes as polymers of amino acids. Deoxyribozymes are not known to exist naturally, but are readily identified by in vitro selection in the laboratory.

Our group has recently reported the first DNA-hydrolyzing deoxyribozyme, 10MD5, which can site-specifically hydrolyze phosphodiester bonds in single-stranded DNA substrates with formation of 3′-hydroxyl and 5′-phosphate products. The 10MD5 deoxyribozyme achieves a rate enhancement of 10^{12} over the uncatalyzed P–O bond hydrolysis. 10MD5 requires both Zn^{2+} and Mn^{2+} and recognizes an ATG^T substrate sequence for catalysis. However, 10MD5 has a sharp pH optimum around pH 7.5, varying the pH by 0.1 pH units in either direction leads to greatly reduced activity. Therefore, 10MD5 was optimized by reselection (in vitro evolution), leading to a set of variants with broader pH tolerance. One of these reselected variants, 9NL27, has only five mutations compared to the original 10MD5 sequence. Extensive biochemical studies of 9NL27 revealed functional compromises among three key characteristics of the initial family of DNA-hydrolyzing deoxyribozymes—pH tolerance, site specificity, and substrate sequence tolerance. As a second consideration, unlike the original 10MD5 deoxyribozyme that requires both Zn^{2+} and Mn^{2+}, the 9NL27 deoxyribozyme functions even with Zn^{2+} alone. Evaluation of the five mutations in the 9NL27 sequence revealed that merely two nucleotide mutations in 10MD5 are sufficient to convert the heterobimetallic 10MD5 deoxyribozyme into a monometallic deoxyribozyme that requires Zn^{2+} alone. Finally, to establish the broad substrate generality of DNA-hydrolyzing deoxyribozymes, comprehensive selections were performed with a key pressure to cleave the DNA substrate at a predetermined site. These efforts led to identification of an initial set of DNA-hydrolyzing deoxyribozymes that recognize merely two substrate nucleotides at the cleavage site (N^G), while retaining Watson-Crick sequence generality beyond those nucleotides. These achievements suggest that comprehensive follow-up experiments will lead to a complete set of DNA-hydrolyzing deoxyribozymes that can collectively hydrolyze any arbitrarily chosen single-stranded DNA substrate.