Development of DNA Catalysts that use Peptide and Protein Substrates

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Deoxyribozymes are catalytic DNA molecules that are identified by in vitro selection. Since the first identification of RNA-cleaving deoxyribozymes in 1994, many deoxyribozymes have been found to catalyze a variety of chemical reactions with significant rate enhancement. Representative reactions catalyzed by deoxyribozymes include phosphodiester bond cleavage and ligation, DNA depurination, and the Diels-Alder reaction. In 2002, a ribozyme was reported that catalyzes the ligation between the N-terminus of an amino group of a peptide as the nucleophile and the 5′-triphosphate of RNA as the electrophile. However, no nucleic acid catalyst (ribozyme or deoxyribozyme) has been identified to catalyze the reaction of an amino acid side chain as a nucleophile.

To address this challenge, our laboratory previously used in vitro selection to identify a deoxyribozyme that catalyzes reactivity of a tyrosine side chain that is covalently embedded within a DNA substrate. The highly preorganized architecture of the deoxyribozyme-substrate complex (left side of figure) presents the amino acid side chain nucleophile in close proximity to the 5′-triphosphate-RNA electrophile, leading to formation of a tyrosine-RNA nucleopeptide linkage. To build upon this initial result, here we sought to identify deoxyribozymes that catalyze reactions of amino acid side chains using a much less preorganized architecture (right side of figure), in which the amino acid side chain nucleophile is presented remotely from the 5′-triphosphate electrophile. We identified the new 10KC3 deoxyribozyme that catalyzes ligation between the tyrosine side chain of a tethered Cys-Tyr-Ala tripeptide substrate and the 5′-triphosphate of RNA. 10KC3 has k_{obs} = 0.006 min^{-1} ($t_{1/2}$ = 2 h) and 93% yield. This finding and related results offer encouragement that deoxyribozymes can be developed to catalyze many reactions of peptide and protein substrates.