DNA-Catalyzed Conjugation of Nucleic Acids and Tyrosine Side Chain

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Deoxyribozymes are single-stranded DNA oligonucleotides identified by in vitro selection that catalyze various chemical reactions. We previously reported DNA catalysts that join tyrosine-containing peptides to RNA in one step by reaction with the hydroxyl group of the tyrosine side chain and the α-phosphate of a 5′-triphosphorylated RNA. Our efforts towards this goal required tethering the peptide substrate to a DNA anchor oligonucleotide. Here, we establish direct in vitro selection using untethered, free peptide substrates. Such a strategy utilizes an azido-modified peptide as the substrate during the selection step and Cu(I)-catalyzed azide-alkyne cycloaddition in the subsequent capture step with an alkyne-modified oligonucleotide to enable PAGE-shift separation of the catalytically active DNA sequences. This approach enables imposition of selection pressure by reducing the peptide concentration and leads to a deoxyribozyme that has a lower apparent K_m value of ~100 μM peptide. In addition, we demonstrate the feasibility of using phosphorimidazolide (Imp) as an alternative electrophile to triphosphate (ppp), noting that Imp can be readily placed at either the 5′-end or 3′-end of either RNA or DNA. This approach provides synthetic flexibility in generation of oligonucleotide substrates for deoxyribozymes and therefore expands the scope of DNA-catalyzed peptide-nucleic acid conjugation. These findings establish a novel and generalizable approach of joining unprotected peptide to nucleic acid in one step using DNA catalysts.