DNA-Catalyzed Reactivity of Amino Acid Side Chains

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Deoxyribozymes are single-stranded DNA molecules that have the ability to catalyze various bioorganic reactions. These DNA enzymes are similar to ribozymes, which are naturally occurring catalytic RNAs known to catalyze important biological reactions, including protein synthesis by the ribosome and RNA splicing by the spliceosome. Deoxyribozymes are not known in nature; therefore, they are identified by a technique called in vitro selection.

Using in vitro selection, our lab earlier identified the Tyr1 deoxyribozyme that catalyzes formation of a phosphodiester bond between a tyrosine hydroxyl and the 5′-end of the RNA (Pradeepkumar et al., *Angew. Chem. Int. Ed.* **2008**, *47*, 1753-1757). In these previous investigations, the tyrosine phenolic OH nucleophile was presented to the 5′-triphosphate electrophile in the context of a three-helix-junction (3HJ) architecture (Fig. 1), which allowed focus on catalysis by DNA rather than binding by DNA of a discrete peptide substrate. However, parallel selection experiments with serine and lysine did not reveal any DNA sequences that catalyze a similar reaction. In the present investigations, we have identified deoxyribozymes (SerB1-SerB4) that catalyze reaction of the serine aliphatic OH nucleophile by expanding the structural context from a single amino acid to an Ala-Ser-Ala tripeptide (Fig. 2) while retaining the 3HJ architecture. Unlike the Tyr1 deoxyribozyme that has very high selectivity for tyrosine over serine; the SerB1-SerB4 deoxyribozymes have rather modest selectivities for serine over tyrosine. We also investigated the positional selectivity of the SerB deoxyribozymes by presenting multiple serine side chains located spatially close to each other. All of the SerB deoxyribozymes show substantial positional selectivity, demonstrating that deoxyribozymes can discriminate between nearby competing nucleophiles.

In summary, we have for the first time identified DNA enzymes that catalyze reactions involving serine side chains, which are much less reactive than the previously used tyrosine side chains. Because of the restriction posed by the overall 3HJ architecture, these deoxyribozymes cannot readily be developed to accommodate free peptide or protein substrates. Therefore, we are currently seeking deoxyribozymes that can utilize free peptides as reaction partners without requiring the 3HJ architecture.

Figure 1. Tyr1 deoxyribozyme (earlier effort)

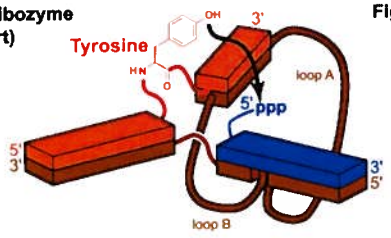

Figure 2. Selection with increased conformational flexibility: SerB1-SerB4 deoxyribozymes (present effort)