DNA-Catalyzed Introduction of Azide at Tyrosine for Peptide Modification

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Synthesis of modified peptides and proteins is essential for understanding the functions of natural post-translational modifications, visualizing protein localization in vivo, modulating therapeutic properties of peptides, proteins, antibodies, and antibody-drug conjugates, and other applications. Tyrosine has often been targeted for synthetic modification on the basis of its electron-rich nature and typically low abundance on protein surfaces. However, current approaches for tyrosine modification rely solely on differential physical accessibilities to discriminate among several reactive sites in the substrate. Therefore, alternative approaches are needed for sequence-selective tyrosine modification. We show that DNA enzymes (deoxyribozymes) can introduce azide functional groups at tyrosine residues in peptide substrates. The introduced azide functional groups can be further modified with useful moieties such as PEG and fluorophores. Using in vitro selection, we identified deoxyribozymes that transfer the 2′-azido-2′-deoxyadenosine 5′-monophosphoryl group (2′-Az-dAMP) from the analogous 5′-triphosphate (2′-Az-dATP) onto the tyrosine hydroxyl group of a peptide, which is either tethered to a DNA anchor or free. Some of the new deoxyribozymes are general with regard to the amino acid residues surrounding the tyrosine, while other DNA enzymes are sequence-selective. We used one of the new deoxyribozymes to modify free peptide substrates by attaching PEG moieties and fluorescent labels. In ongoing work, we are seeking to extend DNA-catalyzed reactivity from peptides to larger protein substrates.