Innovative DNA Analysis Based on Triplex Formation

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A rapid, convenient, and highly sensitive DNA analysis is important to establish tailor-made medications. A main purpose of our work is a development of intelligent nucleic acid analogues to detect a small amount of DNA. The nucleic acid analogue hybridizes with a double-stranded (ds) DNA target in a sequence specific manner to form a triplex structure. This triplex formation triggers off a chemical chain reaction on the dsDNA target. Based on this unique concept, we have synthesized a novel nucleic acid analogue, 5'-amino-2',4'-BNA (5'-amino-2'-*O*,4'-*C*-methylene-bridged nucleic acid), which has a bridged sugar moiety and a P3'→N5' phosphoramidate linkage instead of a phosphodiester linkage. We prepared oligonucleotide probes containing 5'-amino-2',4'-BNA and evaluated their properties. In the presence of the dsDNA target, the oligonucleotide probe formed a stable triplex, and rapid digestion at the P3'→N5' phosphoramidate linkage was observed under mild acidic conditions, while no digestion was detected in the absence of the dsDNA target. By incorporation of a fluorophore and a quencher into the oligonucleotide probe, we successfully achieved sequence-specific and convenient dsDNA sensing. This result indicates that the oligonucleotide probes will play an important role in creating a new technology of DNA analysis. Further chemical modifications of the oligonucleotide probes are now in progress.