Development of Bridged Nucleic Acids for Gene Regulation and Analysis

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In the last decade, increased efforts have been directed toward the development of oligonucleotide-based technologies for genome analyses, diagnostics, or therapeutics. However, natural oligonucleotides have some limitations for application to these technologies, such as insufficient binding affinity and sequence selectivity towords DNA or RNA targets, low stability towards enzymatic degradation. To over come these problems, numerous nucleic acid analogues has been developed to date. One attractive strategy for construction of oligonucleotide analogues having high binding affinity with DNA or RNA targets would be “restriction of a conformational flexibility into a proper shape”. An oligonucleotide has some freely rotating single bonds in its sugar and phosphate backbone. The hybridization step of the flexible oligonucleotide with RNA or DNA is entropically unfavorable owing to fixation of the internal bond rotations. If the oligonucleotide flexibility decreases and its 3D structure is fixed in a suitable form for hybridization beforehand, the entropy loss will decrease significantly. Based on this concept, we have developed a novel bridged nucleic acids (BNAs). One of the BNAs is 2’,4’-BNA (also called Locked Nucleic Acid, LNA) which possesses a methylene linkage between 2’-oxygene and 4’-carbon atoms of ribonucleosides. The 2’,4’-BNA monomer was readily incorporated into oligonucleotides with a standard phosphoramidite protocol on an automated DNA synthesizer. The 2’,4’-BNA oligonucleotides showed unprecedented hybridization ability towards RNA or DNA complements with highly sequence selectivity; therefore, the 2’,4’-BNA oligonucleotides have been widely used as a tool for gene regulation in a living cell. We have also developed various types of BNAs, and some of them were found to be more promising than the original 2’,4’-BNA. Furthermore, additional chemical modification on 2’,4’-BNA oligonucleotides enabled us to obtain a novel gene analysis technology based on a triplex DNA formation.