Abstract

In the first part of this work, a series of site-specific artificial metallonucleases (AMNs) was developed conjugating clamped-phenanthroline (Clip-Phen) copper complexes to triplex-forming oligonucleotides (TFOs). Several synthetic routes were explored for the synthesis of the TFO-AMNs hybrids, all sharing a copper-catalyzed alkyne-azide cycloaddition (CuAAC) reaction as the key step. As a consequence, building blocks for enzymatic or chemical synthesis of oligonucleotides (ONs) containing clickable groups, or already conjugated to the Clip-Phen ligand via CuAAC, were prepared.

Two new alkynyl-linked nucleoside-5'-*O*-triphosphates (dNTPs) were designed and developed in order to obtain an efficient polymerase incorporation of clickable alkynyl-tethers into ONs and, at the same time, enhance the efficiency of CuAAC reactions on modified DNA. The relative 3'-*O*-phosphormaidites were also prepared in order to insert the same alkynyl-linkers into ONs via solid-phase synthesis.

The AMN was linked at the 5'- or 3'-ends or in the middle of the TFO stretch, using diverse likers. The hybridization of all the synthesized TFOs with a target DNA duplex was studied. Finally, an extensive study of cleavage efficiency and specificity of the TFO-AMN conjugates towards the target DNA was performed, exploring the influence of the hybrid nature and reaction parameters.

In the second part of this work, a systematic study of competitive polymerase incorporation of 5-substituted pyrimidine and 7-substituted 7-deazapurine dNTPs, in the presence of their natural counterparts (natural dNTPs) was performed. The base-modified dN^RTPs were incorporated into ONs by competitive primer extension (PEX) or polymerase chain reaction (PCR), in different ratios with natural dNTPs. A study of enzymatic kinetics of single-nucleotide incorporation was performed to explore the substrate affinity to the Bst DNA polymerase and incorporation rate of all base-modified dN^RTPs.

All studied dNTPs were successfully incorporated into DNA in the presence of natural dNTPs. 7-deazapurine dNTPs bearing π-electron-containing substituents, and 5-phenyl pyrimidine dNTPs, were found to be even better substrates of the Bst polymerase than their natural counterparts.