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Aspects of Antisense and Antigene Chemistry of Oligonucleotides Tethered to Intercalators

Dissertation in Bioorganic Chemistry to be publicly examined in the lecture hall B 21 (corridor A2, floor 1) at the Biomedical Center, Uppsala University, on October 5, 2002, at 10:00 a.m., for the Degree of Doctor of Philosophy in Bioorganic Chemistry. The examination will be conducted in English.

ABSTRACT

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Synthetic and physicochemical studies on appropriately functionalized ODN-conjugates have been performed to evaluate their abilities to act as antisense agents against RNA or as intramolecular DNA cross-linking agents. Intercalating aromatic systems [phenazine (Pnz), dipyridophenazine (DPPZ)] and metallointercalators such as $Ru^{2+}(phen)_2(DPPZ)$ and $Ru^{2+}(tpy)(DPPZ)L$ [where L = chemically or photochemically labile ligand, phen = phenanthroline, tpy = terpyridine], which are covalently tethered to the oligodeoxynucleotides (ODNs), have been chosen for this purpose. The ODN-conjugates were typically prepared by automated solid phase synthesis using phosphoramidite building blocks, or on solid supports, both functionalized with the chromophore groups. The photosensitive metal complex, $Ru^{2+}(tpy)(DPPZ)(CH_3CN)$, has been incorporated by post-synthetic coupling to the amino-linker modified ODNs via an amide bond. The intercalating ability of the tethered chromophores gave enhanced stability of the duplexes and triplexes formed with ODN-conjugates and their complementary targets: DNA, RNA, or double-stranded DNA. The conjugation of DPPZ chromophore to ODN (at 3', 5' or at the middle) led us to incorporate $Ru^{2+}(phen)_2(DPPZ)$ through the DPPZ ligand, for the first time. The corresponding (Ru^{2+}-ODN)•DNA duplexes showed dramatic stabilization (ΔT_m = 19.4 – 22.0ºC). The CD and DNase I footprinting experiments suggest that the stabilization is owing to metallointercalation by threading of the $Ru^{2+}(phen)_2$ moiety through the ODN•DNA duplex core, thus "stapling" the two helical strands from the minor to major groove. On the other hand, $Ru^{2+}(tpy)(DPPZ)(CH_3CN)$-ODN conjugates represent a new class of oligonucleotides containing the photoactivatible Ru^{2+} complexes, which can successfully crosslink to the complementary strand. The mechanism of cross-linking upon photoirradiation of [$Ru^{2+}(tpy)(DPPZ)(CH_3CN)$-ODN]•DNA involves *in situ* conversion to the reactive [$Ru^{2+}(tpy)(DPPZ)(H_2O)$-ODN]•DNA which are subsequently cross-linked through the G residue of the complementary DNA strand. All starting materials and products have been purified by HPLC and/or by PAGE and subsequently characterized by MALDI-TOF as well as ESI mass spectroscopy. Terminal conjugation of the planar Pnz and DPPZ groups through the flexible linkers were also shown to improve thermal stability of the ODN•RNA hybrid duplexes without alteration of the initial AB-type global helical structure as revealed from CD experiments. As a result, RNase H mediated cleavage of the RNA strand in the intercalator-tethered ODN•RNA duplexes was more efficient compared to the natural counterpart. The RNase H cleavage pattern was also found to be dependent on the chemical nature of the chromophore. It appeared that introduction of a tether at the 3'-end of the ODN can be most easily tolerated by the enzyme regardless of the nature of the appending chromophore. The tethered DPPZ group has also been shown to chelate Cu^{2+} and Fe^{3+}, like phenanthroline group, followed by the formation of redox-active metal complex which cleaves the complementary DNA strand in a sequence-specific manner. This shows that the choice of appropriate ligand is useful to (i) attain improved intercalation giving T_m enhancement, and (ii) sequence-specifically inactivate target RNA or DNA molecules using multiple modes of chemistry (RNase H mediated cleavage, free-radical, oxidative pathways or photocross-linkage).

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