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CYSTAMINE-MODIFIED RNA OLIGONUCLEOTIDES: SYNTHESIS AND APPLICATION IN CROSS-LINKING AND CONJUGATION VIA DISULFIDE BOND

Abstract

The development of the method of DNA and RNA oligonucleotide chemical synthesis revolutionized the world of biology, biochemistry, and related research areas. These short fragments of nucleic acids allowed for characterization of multitude of interactions and mechanisms which comprise a basis of living organisms that beforehand were impossible to study. Nowadays, oligonucleotides are fundamental tools of molecular biology, diagnostics, and- increasingly- medicine.

Chemically modified oligonucleotides can be very useful in biomolecular research playing vital role in biomolecule intractions. There are numbers of known modifications of different properties and applications and with the current state-of-the-art, it is possible to devise an entirely tailored functionalization method. One of the possible modifications is the introduction of linkers into the nucleic acid chain that allow covalent bonding with another molecule, e.g. fluorescent label or protein, which is owing to the functional groups present in linkers' structure. Diverse types of linkers of different functional group reactivity are available, therefore the choice depends on the application and its function.

Oftentimes linkers are introduced at terminal positions of the oligonucleotide, however such solution has limited applicability, especially if a specific, precise position of the linker within the chain is required. The main goal of my research was the development of a method that would allow for obtaining stable RNA oligonucleotides modified with 2,2'-dithiobisethylamine (cystamine) linker at chosen phosphodiester bond within the nucleic acid chain (P-cystamine RNA). Cross-linking or conjugation via cystamine is reversible, which can be beneficial in certain applications. Thiol, which is a functional group of such modified oligonucleotide undergoes oxidation with a thiol group present in another molecule to form a disulfide bridge. This bond can be in turn reduced resulting in the liberation of the two molecules.

To obtain P-cystamine RNA I have used a combination of two synthetic approaches: the phosphoramidite method and the H-phosphonate method. Nucleic acid modification was achieved through condensation of a chosen nucleoside *H*-phosphonate and subsequent oxidative coupling of cystamine (amination) in Atherton-Todd reaction, which yielded P-cystamine bond. Initial attempts to obtain such modified RNA using only ribonucleotide

H-phosphonates had failed since the product underwent cleavage. One of the parts of my work focused on the identification of the products of hydrolysis to suggest the mechanism of the cleavage. In order to obtain a stable P-cystamine RNA, I have decided to introduce ribonucleotide analogs at the site of linker attachment. For that purpose, I used 2'-deoxyribonucleotides and 2'-deoxy-2'-fluororibonucleoitde *H*-phosphonates. Such point modifications yielded stable oligonucleotides with minimal influence on the overall geometry of the molecule. Next, I optimized reaction conditions by examining the impact of such variables as *H*-phosphonate condensation time, type of condensing agent, or oxidative coupling reaction conditions.

Yet another goal of my project was a study of the stereochemistry of reactions and products. At the first stage I focused on obtaining simplified dinucleotide models, which allowed me to identify the influence of heterocyclic base preceding the modification as well as C2' substituent of the *H*-phosphonate. Additionally, I examined the stereochemistry of P-cystamine RNA in order to investigate whether further neighborhood can influence the stereochemistry of the product.

Further, I focused on application of modified oligonucleotides in cross-linking reactions. I optimized reaction conditions and carried out a series of experiments of intermolecular cross-linking of P-cystamine RNA with both complementary and non-complementary sequences. I have also attempted to obtain covalently stabilized RNA hairpins by intramolecular cross-linking. I expanded the studies by using additional homobifunctional linkers with maleimide functional groups.

One of the assumed applications of P-cystamine oligonucleotides is their use in structural and mechanistic studies of proteins. Modified oligonucleotides which I synthesized were used in conjugation with RNase H1. In collaboration with the Laboratory of Protein Structure of the Institute of Molecular and Cell Biology, we obtained stable complexes of RNase H1 mutants with modified DNA:RNA substrate, which proved intermolecular interactions of the two species. Obtained complexes can be used for further structural studies.

To summarize, the methods I have developed and obtained results can serve in future research on the applicability of P-cystamine RNA as molecular biology tools, particularly in studies of RNA-protein interactions.