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Therapeutic potential of guanine-rich oligonucleotides.
Structural and functional characteristics.

ABSTRACT

Understanding the information contained in genome or transcriptome sequences and the therapeutic management of cellular processes are the greatest challenges of our time. In the case of genetically determined diseases, knowledge of the sequence of the pathogenic gene is the basis for the design of drugs targeting the changed gene or a protein derived from it. In recent years, the results of numerous studies have indicated the key role of quadruplexes in many biological processes. The quadruplex structures are formed from guanosine rich sequences, which under appropriate conditions, form stacked layers of guanine tetrads. Putative quadruplex sequences are commonly found in the human genome and transcriptome. Numerous diagnostic and therapeutic tools have been proposed, the mechanism of which is based on the induction and stabilization of quadruplex structures. For example, quadruplexes have proved to be attractive targets in anti-cancer therapy and in the treatment of neurodegenerative diseases. One way to regulate the expression of a pathogenic gene, in which quadruplexes play a key role, is to use appropriate quadruplex specific G4-ligands.

My research was concerned with the properties of guanine-rich bifunctional antisense oligonucleotides and examining the possibility of using them to selectively recognize target RNA sequences that differ by only one nucleotide residue. Such an approach is based on the synergistic effect of two nucleic acid structural elements, called the duplex and quadruplex domains. This approach enables the recognition of a point mutation (U→G) by the formation of different secondary structures after attaching the antisense oligonucleotide to the target RNA – either a duplex-quadruplex hybrid (**DQH**) structure or a duplex with unstructured ends (**Dss**). Using various experimental techniques, such as NMR, UV, CD spectroscopy or polyacrylamide gel electrophoresis, I investigated the properties of these types of structure under various conditions and determined the influence of non-nucleotide chemical modifications (abasic residues, aliphatic linkers) on the stability and structure of the studied **DQH** and **Dss** complexes. I showed that a G4-ligand covalently attached to an antisense oligonucleotide binds selectively to the quadruplex domain in a duplex-quadruplex hybrid structure. Such a G4-ligand covalently bound to the antisense oligonucleotide can slow down the quadruplex unwinding process in the cell and also serve as a selective fluorescent marker for the detection of bimolecular quadruplexes.

In cooperation with the Laboratory for the Analysis of Subcellular Structures IBCH PAS, I have shown that the oligoribonucleotides designed by me, silence gene expression more effectively than classic antisense oligonucleotides, which only create duplexes with the target RNAs. The ability to recognize a single nucleotide change in the target sequence by an antisense oligonucleotide has great therapeutic potential. For example, in patients with non-small cell lung cancer a gene mutation is often diagnosed that results in a sequence change in the mRNA fragment from GGGCUGG to GGGCGGG, which is a potential target for therapy using bifunctional oligonucleotides rich in guanosine residues.