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Investigation of the rotor effect of modified 8-oxo-adenosine as part of DNA duplexes

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Motivation and Aim: The detection of single nucleotide polymorphisms (SNPs) in nucleic acids is an urgent task of modern biomedicine. One of the approaches allowing their detection is the creation of highly specific fluorescent oligonucleotide probes. The main goal of our research is to test the physicochemical and structural properties of DNA duplexes containing a previously unexplored fluorophore: 8-oxo-N6-((3-methyl)-2-benzothiazolone)-2′-deoxyadenosine (X). The main purpose of my study is to gain an understanding of whether this fluorophore can be used to search for SNPs.

Methods and Algorithms: The fluorescence properties of X in the free state, as part of oligonucleotides and their duplexes, were determined by quantum yield determination using a standard. The hybridization properties of X were evaluated by thermal denaturation and computer modeling methods.

Results: The physicochemical properties of (8-oxo-6-(3-methyl-2-benzothiazolone)-adenosine and oligodeoxyribonucleotides containing this fluorescent nucleoside were investigated as follows. The fluorescence quantum yield value of the nucleotide was 1.2 ± 0.2 % fluorescence. The value of quantum yield of oligonucleotides containing X increased from 9 to 22 times. When forming duplexes with DNA, changes in the value of fluorescence the quantum yield are multidirectional, depending on the nucleotide environment of the fluorophore. Our hypothesis was not confirmed since we did not see significant changes in X fluorescence during duplex formation. The results of my study indicate that as part of duplexes, the fluorophore cannot become brighter because its rotation around the C6-N bond is too mobile regardless of duplex composition. There is a need to redesign or restructure our research in a different way.

The thermal stability of complexes with single-nucleotide mismatches increases in the series of bases G > T > C; destabilization of complexes is observed only in the case of the location of A opposite the modification. In complexes in which the modified nucleotide is surrounded by pyrimidine bases and adenine is opposite the modification, the greatest destabilization of DNA duplexes is observed.

Since we tested a large number of X-containing sequences, we hypothesize that changing the design of a conventional fluorophore will help improve its fluorescent properties in DNA duplexes.

Conclusion: Today, a large number of fluorophores have been developed; however, debate continues about the best strategies for the design and improvement of the properties of such molecules.

Most studies in the field of the development and research of fluorophores have only focused on creating simple and cheap molecules to synthesize. But the results of previous

studies have proved inconclusive because they strongly disturb the DNA structure or have insufficient fluorescence intensity. To sum up, it is possible to confirm the existing theory that the quantum yield of oligonucleotides and their duplexes increases significantly in comparison with the free nucleoside.

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