Секция «Биофизика и нанобиотехнологии»

Development of Binary Deoxyribozyme sensor modified with LNA for the detection of dsDNA

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Binary Deoxyribozymes sensors (BiDz) are highly sensitive novel diagnostic tool. As it was previously described [1], these versatile molecules can be tailored to a variety of targets [2]. Dividing the functional roles between several components of the sensor allows for the fine tuning of the sensor's characteristics, including selectivity. It consists of a 10-23 Deoxyrbizoyme (Dz) core that split into two fragments to form halves in addition to the analyte-binding arms BiDza and BiDzb. The BiDza and BDzb hybridize to a complementary fragment in nucleic acid analyte and form a catalytically active Dz core, which can cleave a specifically designed fluorophore and quencher labeled reporter substrate, thus producing a fluorescent output. The signal is accumulated over time due to the catalytic turnover. The BiDz probes act perfectly well in case of ssDNA but their performance in dsDNA is restrained. Hybridization of a probe to dsDNA is energetically unfavorable process. Indeed, when a DNA probe hybridizes to a DNA duplex, the number of newly formed and the lost base pairs is roughly equal. This is because a fragment dsDNA opposed to the bound probe remains unpaired. The entropy change does not favor the association reaction either. Therefore, ΔGo of probe-dsDNA complex formation is positive.

When modified with Locked Nucleic Acids (LNA) [3], oligonucleotides exhibit an increased affinity and specificity towards its target. There is no clear guideline in literature on where and how many LNA modifications should be introduced to the oligonucleotides. In an attempt to fill this gap, we set out to investigate the effect of number and position of LNA modification that should be introduced to the analyte-binding arms of the binary deoxyribozyme sensors in order to enhance its ability to detect dsDNA.

We tested a series of different LNA-modified and unmodified BiDz to examine the effect of position and number of LNA substitutions on the BiDz/dsDNA complex stabilization. We found the alteration in BiDz sequence between LNA and regular DNA generated the best dsDNA invasion efficiency. We also confirmed that the BiDz is also highly selective, since it was able to detect and differentiate between different SNPs. This result will be later applied for nd-FISH development.

Авторы исследования благодарны Министерству образования и науки Российской Федерации № FSER-2022-0009

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