DEVELOPMENT OF FLUORESCENT HYBRIDIZATION PROBES FOR HIGHLY SELECTIVE DETECTION OF DOUBLE STRANDED DNA ANALYTES AT 37ºC AND ROOM TEMPERATURE

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Introduction. Hybridization of a probe to ssRNA or ssDNA analytes is favorable due to the favorable enthalpy of Watson-Crick base pair formation. On the other hand, entropy change upon formation of such complexes is always unfavorable. 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, ΔG of probe-dsDNA complex formation is positive. The same is true when double stranded probe hybridizes to analyte.

Protein-free assays that utilizes RNA-cleaving deoxyribozymes (DNAzymes or Dz) for signal amplification have been developed [1]. For the diagnostic purposes, a Dz can be split in two fragments to form a binary Dz sensor. The two fragments hybridize to a complementary region 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.

Main part. We argue that analysis of dsDNA requires neither probe excess nor denaturing conditions. Analysis of dsDNA is possible by accurate DNA hybridization sensor designed according to the specific rules. Negative Gibbs energy can be achieved due to the formation of additional base pairs in the complex formed between a multicomponent sensor and dsDNA target. Indeed, if a set of short oligonucleotide staples (SOS) unassociated or poorly associated with each other hybridize to dsDNA the sum of base pairs formed can exceed those broken. The major challenge in creating such a multicomponent sensor is to achieve only marginal association of SOS in the absence of dsDNA, because SOS are intrinsically self-complementary.

To test the hypothesis, we designed two sets of hybridization probes based on DNAzymes and Locked Nucleic Acid (LNA) modifications [2]. LNA contains a methylene bridge between the 2´O and 4´C atom in the ribose ring to form a bicyclic ring. The bridge locks the sugar moiety in an N-type (C3'-endo) sugar ring conformation resulting in enhanced hybridization properties. As a result of this conformation, the backbone of LNA is preorganized which leads to energetically favorable duplex formation, in part through increased base stacking interactions.

The first set is DNAzymes- and LNA- based SOS. The sensor SOS invade the dsDNA and form a probe-dsDNA complex due to the enhancing properties of LNA modifications. Once the dsDNA is invaded, the catalytic core of the Dz is formed (i.e., become catalytically active to cleave the fluorophore and quencher labeled reporter substrate). The second set is Triplex-Forming oligonucleotides (TFO) based DNAzyems. Triplexes are generated intermolecularly when the third strand originates from a different DNA molecule. The third strand oligonucleotide recognizes a purine-rich sequence and binds the dsDNA through the major groove. LNA modification were also introduced to the sensor. As a target, we chose a fragment of MYC Gene - MYC Proto-Oncogene, BHLH Transcription Factor for both sets of hybridization probes.

Conclusion. The first variant of hybridization probes was developed and tested. The probe was tested on 45 nt long synthetic dsDNA. The fluorescent signal was detected after 24h of incubation at 37ºC. The detected signal indicates a dsDNA invasion and therefor the formation of the catalytically active Dz core. Further development of the probe for the detection of dsDNA analyte will be carried on along side of testing the second variant of hybridization probes.

List of references:

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