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Hemoglobin-binding 2'-F-modified RNA aptamers: structure studies and analytical application

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Motivation and Aim: DNA and RNA aptamers specifically recognize various molecular targets due to the formation of a specific three-dimensional structure. Such unique characteristics of aptamers as stability, ease of synthesis and modification as well as low immunogenicity and toxicity promoted the development of aptamer-based diagnostic and therapeutic tools. Particularly, aptamers are widely used as molecular recognizing elements for biosensors (also called aptasensors). Nowadays, there are plenty of electrochemical, fluorescent, colorimetric, and surface plasmon resonance aptasensors for the detection of a wide target spectrum [1]. Colorimetric detection systems are quite simple and easy to perform, provide reliable results, and suits well for point-of-care testing. Enzyme-linked immunosorbent assays (ELISA), which rely on plate reader colorimetry, are routinely used for laboratory diagnostics. Nevertheless, this method has its drawbacks determined by the use of antibodies: the high cost of diagnostic kits, their relatively short shelf-life, and lot-to-lot variability, which hampers the long-term studies [2]. The development of ELISA-like systems based on aptamers is a very promising approach to biomarker detection. In the present study, we aimed to select hemoglobin-binding 2'-F-modified RNA aptamers, analyze their structure and demonstrate their possible application as biorecognizing elements of microplate colorimetric assays.
Methods and Algorithms: We utilized SELEX method for *in vitro* selection of hemoglobin-binding aptamers using combinatorial library of 2'-fluoro pyrimidine modified RNAs. The final enriched library was sequenced using the NGS platform MiSeq (Illumina). A series of G-rich individual aptamers were chemically synthesized and screened for their binding activity against human hemoglobin. For detailed analysis of G-quadruplex formation, we employed CD spectroscopy, enzymatic structure probing and G-quadruplex specific fluorophore binding assays. We also performed enzyme-linked aptamer-based microplate assays both in direct and sandwich formats for hemoglobin detection.
Results: We selected guanine-rich 2'-fluoro pyrimidine modified RNA aptamers against human hemoglobin. Three 2'-F-RNA aptamers contained G-stretches, the analysis of their secondary structures by the Vienna algorithm revealed a possibility of G-quadruplex formation. The formation of parallel quadruplexes for these aptamers was confirmed by a combination of experimental assays. We established an aptamer-based colorimetric detection of human hemoglobin in microplate assays and demonstrated that quadruplex-forming 2'-F-RNA aptamers are suitable for this detection in direct and sandwich formats. We also revealed that the sandwich-type detection system based on two aptamers could generate a nonspecific colorimetric signal, probably due to complex formation between two aptamers used in the assay.

Conclusion: In the present study, we selected hemoglobin-specific G-rich modified RNA aptamers and confirmed the formation of G-quadruplex for these aptamers. We successfully used obtained aptamers for hemoglobin detection in microplate colorimetric assay.

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