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CREATION OF CIRCULAR ANTISENSE OLIGONUCLEOTIDES: A NOVEL STABILIZATION STRATEGY FOR GENE THERAPY AGENTS

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Введение. Therapeutic nucleic acids, such as antisense oligonucleotides (ASOs), have emerged as powerful tools for modulating gene expression, offering significant potential for treating various genetic disorders and diseases. However, their clinical application is often hindered by challenges such as low stability, poor cellular uptake, and susceptibility to nuclease degradation. Circular ASOs, characterized by their closed-loop structure, present a promising alternative due to their enhanced resistance to nucleases and increased stability in biological environments.

Основная часть. Circular oligonucleotides are among the most extensively studied topological modifications of synthetic DNA. Their unique closed-loop configuration provides several advantages over linear oligonucleotides, including increased resistance to exonucleases, which are enzymes that degrade nucleic acids by cleaving them from the ends. This resistance is particularly crucial for therapeutic applications, as it extends the half-life of the oligonucleotides in vivo, allowing for more sustained gene silencing effects [1]. Additionally, the lack of free ends in circular ASOs prevents rapid degradation, making them more stable in cellular environments compared to their linear counterparts [2].

To create circular ASO, we employed a novel approach based on the assembly of a double-crossover (DX) tile, a DNA nanostructure first introduced in 1993 [3]. The DX tile consists of five DNA strands that form two parallel helices connected by two crossover points. This structure was used to bring the 5' and 3' ends of the ASO into close proximity, enabling their chemical ligation. We designed several modifications of the ASO ends, including phosphate and hydroxyl groups, to optimize the ligation process and ensure efficient circularization.

The assembly of the DX tile involved mixing the oligonucleotides in a buffer containing imidazole-HCl and $MgCl_2$, followed by annealing at 95°C and gradual cooling to room temperature. This process allowed the formation of the DX tile structure, which served as a template for the circularization of the ASO. For the chemical ligation step, we used 1-cyanoimidazole and $MnCl_2$, which facilitated the formation of a phosphodiester bond between the 5' and 3' ends of the ASO, resulting in a circular structure.

The successful formation of circular ASOs was confirmed using gel electrophoresis. Both native and denaturing gels were employed to analyze the assembly of the DX tile and the ligation products. The results demonstrated the efficient formation of circular ASO, as evidenced by the distinct bands corresponding to the circularized products. This confirmed the effectiveness of the DX tile-based approach for creating circular ASO.

Выводы. The development of circular ASOs using DX tile assembly and chemical ligation represents a significant advancement in the field of gene therapy. Circular ASOs exhibit enhanced stability and resistance to nucleases, making them highly suitable for therapeutic applications. This study not only demonstrates the feasibility of creating circular ASOs but also aims to highlight their potential as a powerful tool for gene knockdown. Future research will focus on investigating the self-delivery of circular ASOs to target cells and evaluating their gene knockdown efficacy *in vitro*.

Список использованных источников:

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