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Optimization of Aptamer AS1411: An Effort to Increase Binding Efficiency to Nucleolin, an Overexpressed Protein on Cancer Cells

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ABSTRACT

This project aimed to manipulate DNA (deoxyribonucleic acid) aptamer AS1411, a short single-stranded oligonucleotide currently being developed to improve chemotherapy's target cell specificity. As this aptamer binds explicitly to nucleolin, an overexpressed protein on the surface of cancer cells, chemotherapy damage to surrounding tissue may be lessened. This study modified the AS1411 DNA aptamer, which was named AS1411-N12, by adding 12 nucleotides to the 3' and 5' ends, forming a "flap" structure. The edition of said flap is attributed to theory that the increased mass will allow for tighter binding. This modification was hypothesized to further improve the DNA aptamer's binding efficiency to the nucleolin protein expressed on cancer cells. Binding reactions occurred between DNA aptamers (AS1411 and AS1411-N12) and nucleolin samples. The resulting solutions were processed using micro-centrifugal filters, which separated small unbound single-stranded DNA aptamers from bigger unbound proteins and the DNA-Nucleolin complexes. Measured absorbance of the unbound filtered DNA aptamers were analyzed to compare binding efficiencies of the modified aptamer vs. the control. The average absorbance through 3 trials of the control AS1411 DNA aptamer was 1.907 at 260 nm, while the average absorbance through 3 trials was 1.364 at 260 nm. Through Beer's Law, the unbound DNA control concentration was 146.6 μM while the modified DNA aptamers was 54.17 μM. This modification was highly effective as it yielded a 63% change in absorbance showing a drastic decrease in the amount of DNA aptamer left in solution. The modified DNA aptamer was significantly more effective in binding to its target protein. When attached to chemotherapy, AS1411-N12 will have a higher affinity to Nucleolin, improving cancer treatment.

Keywords

Aptamer, nuclear proteins, nucleolin, AS1411, AS1411-N12, spectrophotometry, DNA.

Introduction

Cancer treatment faces a critical challenge: delivering therapies with maximum precision and efficacy while minimizing side effects. For example, chemotherapy is a highly nonselective process as a result of a failure of DNA aptamers or other drug delivery systems to bind tightly to the proteins expressed on cancer, causing the chemotherapy to go "rogue" in the bloodstream, leading to intense side effects. These include side effects such as pain, hair loss, fatigue, kidney problems, weight changes, and more [1]. This project aims to manipulate aptamer AS1411, a short single-stranded oligonucleotide that is a delivery method for chemotherapy, by addition of certain nucleotides, in order to make a "flap", improving binding efficiency to nucleolin, an overexpressed protein on the surface of cancerous tumor cells [2-4], and hopefully minimize the amount of rogue chemotherapy in the bloodstream.

Aptamer: Aptamers are oligomers of artificial ssDNA (single-stranded DNA), RNA (ribonucleic acid), XNA (xenonucleic acid) or peptide that bind to a specific target molecule, or family of target molecules.

Materials

This project is centered around the binding reaction of aptamer AS1411 (IDT) [5] and Nucleolin (FC Tag: NUL-H5253). Two sets of aptamers are used: the stock AS1411 and a modified version,

AS1411-N12 [6]. The protein is resuspended with deionized water 250 µL. A Microcentrifuge administers the binding reaction. Amicon filters (30 kDa (kilodalton)) separate the bound complex and the unbound proteins from the unbound aptamers [7]. A NanoDrop (SKU: 28127 Thermo Fisher Scientific) is used to read and analyze the unbound DNA to determine concentration. Clean, dry, lint-free lab wipes handle the Nanodrop sensor.

Methodology

Stage 1: *Preparation*

The first step is to obtain the AS1411 aptamer (IDT) [5], an ssDNA oligonucleotide, and the Nucleolin protein (Acrobio). This is the control sample, which is an unmodified stock version. The Aptamer will arrive in a 100 µM solution of IDTE. Next, the protein nucleolin, which is overexpressed on cancerous tumors, is obtained, and the aptamers will bind to it [8]. The nucleolin arrives lab lyophilized and reconstituted with deionized water 250 µL and then further diluted with 1.25 mL PBS. (phosphate buffered saline).

Stage 2: *Modification of AS1411*

AS1411 is an aptamer with a unique g-quadruplex structure, which provides an opportunity to improve the surface area-to-volume ratio. 12 Nucleotides are placed at the 3' and 5' ends to create a "flap." This flap aims to increase binding efficiency by adding additional contact points to the biological receptor (Nucleolin). The enhanced aptamer, named AS1411-N12, reflects the original AS1411 with the addition of 12 nucleotides on each end [6]. AS1411-N12 is ordered from IDT [5] (Integrated DNA Technologies), matching the specifications of the control.

Stage 3: *Binding Reaction*

Next, 0.25 mL of the protein and DNA are combined and directly incubated at 4°C (degrees celsius) for 1 hour. Two different binding reactions are conducted: a control (AS1411 and Nucleolin) and an experimental (AS1411-N12 and Nucleolin). Both reactions follow the same procedure. Three tests per control and modified DNA aptamer will be carried out.

Stage 4: *Filtration*

Amicon Ultra centrifugal filters collect filtrate via the microcentrifuge for both the control and experimental with equal volumes. These filters consist of two tubes, one collecting filtrate while the other recovers the concentrated sample. This is used to filter the solution, separating, based on physical size, the small, unbound aptamer from the aptamer-protein-bound complex [7]. This was centrifuged at 13,500 RPM (revolutions per minute) for 20 min at 22°C.

Stage 5: *NanoDrop/Data*

DNA is extracted and the NanoDrop is set to the ssDNA tab-measuring the absorbance of unbound single-stranded DNA (aptamer). 5 µL (microliter) of the blanking solution, derived from the resuspension solute, is placed on the sensor—sanitized to prevent contamination. Trials use 5 µL of the unbound DNA concentrated sample. The NanoDrop provides graphs based on the Ultraviolet-visible spectrum. Using Beer's Law, the concentration of unbound DNA is calculated to assess the aptamer's binding efficiency [9]. The concentration of DNA found in the sample indirectly relates to the binding capability of AS1411. The lower the concentration of DNA found infers that a larger amount of DNA was bound to the protein.

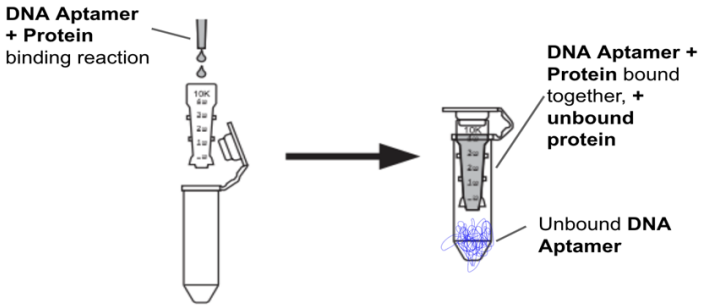

Figure 1: Methodology of collecting unbound DNA Aptamer.

Results

The average absorbance through 3 trials of the control AS1411 DNA aptamer was 1.907 at 260 nm, while the average absorbance through 3 trials of AS1411-N12 was 1.364 at 260 nm. Through Beer's Law, the unbound DNA control concentration was 146.6 µM while the modified DNA aptamers was 54.17 µM [9]. This modification was highly effective as it yielded a 63% change in absorbance, showing a drastic decrease in the amount of DNA aptamer left in solution.

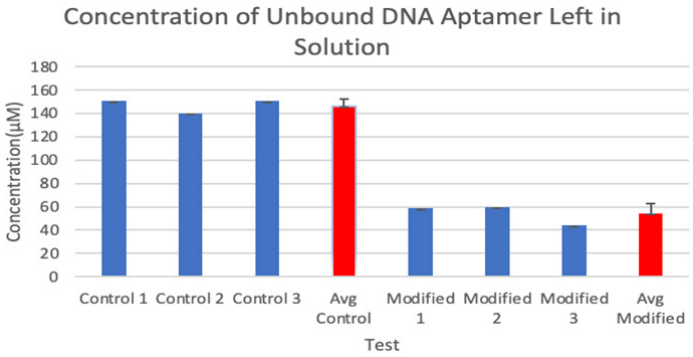

Figure 2: Concentration of unbound DNA Aptamer.

Analysis

The results show that the AS1411-N12 had a higher binding affinity, which would lead to fewer total unbound strands. This modification proved to exponentially increase the binding efficiency to the protein. Clearly, since significantly less DNA aptamer is left in the filtered solution, this means that a significantly greater amount has remained bound to the protein, and not filtered through. These non-deviating results very conclusively and clearly demonstrate how the new, modified, DNA aptamer is significantly more effective and efficient in binding to the nucleolin protein found on cancer cells, improving treatment for patients indirectly by 63% (the percent change in concentration between the original

and modified DNA aptamer) [2,6].

Discussion

The results clearly show how adding the "flap" enhanced binding efficiency. The increased surface area to volume ratio has created additional points of contact for the aptamer to latch onto the protein [6,10]. Just looking at absorbance, the average of the control was 1.907 (10 mm) at 260 nm (nanometer) while the modified was 1.364 (10 mm) at 260 nm. This modification reduced the amount of unbound DNA strands by 63%. Through this lowered concentration due to the higher binding affinity of AS1411-N12, treatment in the future can be less physically demanding, reducing the volume of side effects. Through the six tests performed, 3 control and 3 experimental, the data stayed consistent. The control had a standard deviation of 5.98, while the experimental had a standard deviation of 8.91. This shows how the control data stayed extremely close to the average concentration which was 146.6 µM. Similarly, the experimental data stayed consistent with the average concentration of 54.17 µM. This consistency exemplifies how the modified flap, consisting of 12 nucleotides on each end, was not a fluke result and improved the concentration of bound complexes. This concentration was lower than the control by 92.43 µM. With these consistent results, further testing should be conducted in different environments to monitor performance [11]. Clearly, since significantly less DNA aptamer is left in the filtered solution, this means that a significantly greater amount has remained bound to the protein, and not filtered through. These non-deviating results very conclusively and clearly demonstrate how the new, modified, DNA aptamer is significantly more effective and efficient in binding to the nucleolin protein found on cancer cells [12,13],

Macromolecule	kDa	Filter kDA	Pass through (YES/NO)
AS1411 (Unbound)	8.58	30kDa	YES
AS1411-N12 (Unbound)	16.5	30kDa	YES
Nucleolin (Unbound)	102.9	30kDa	NO
DNA-Protein Complex (control)	111.48	30kDa	NO
DNA-Protein Complex (modified)	119.4	30kDa	NO
*Single Nucleotide is 0.33 kDa			
*These Cellulose filters are redcomened to use a membrane with a kDa at least two times smaller than the molecular weight of the protein solute that one intends to concentrate.			
*kDa is a measure of Molecular weight			

Figure 3: Molecular weight comparison chart.

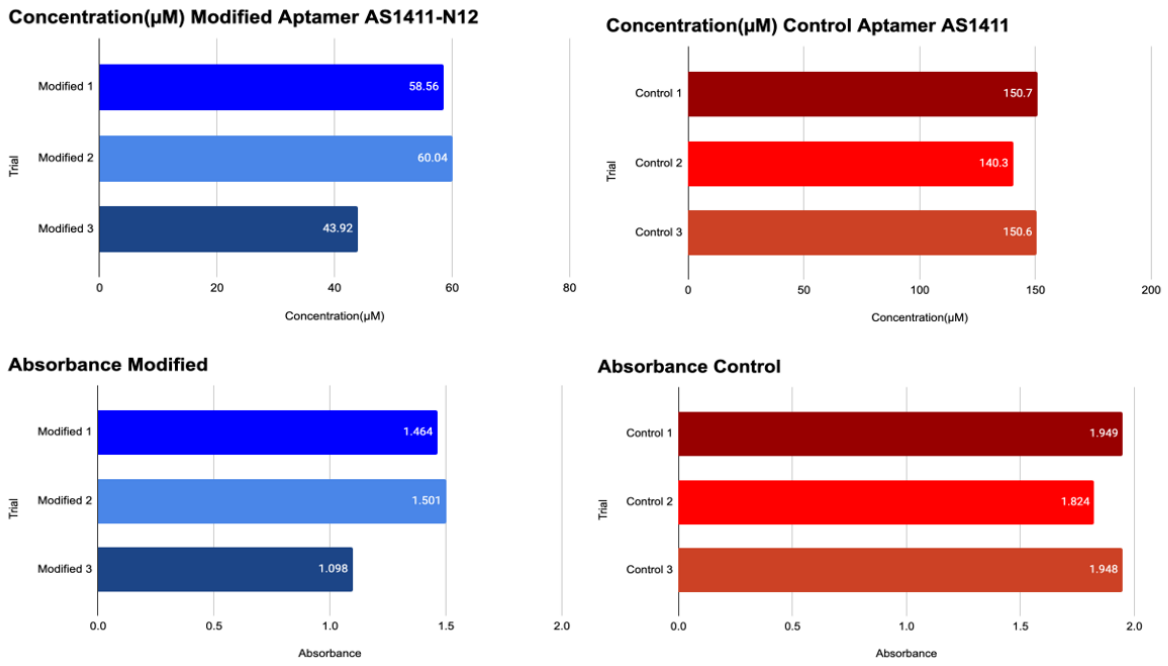

Figure 4: Measured absorbance and concentration of control and experimental groups.

improving treatment for patients indirectly by 63% (the percent change in concentration).

Possible Errors

DNA and protein have specific ranges including pH, temperature, etc., which will alter their structure, disabling the intended function [14]. When Nucleolin is exposed to uninhabitable conditions, hydrogen bonds will break, leading to a changed quaternary structure- disabling the function. If the AS1411 DNA aptamer is exposed to uninhabitable conditions, the phosphodiester bonds connecting the pentose sugar and the phosphate group from one nucleotide to another would break. No hydrogen bonds would break because it is a single strand of DNA. In the PCR (polymerase chain reaction) process at IDT [5], they could have introduced a mutated version and replicated that, which would have heavily skewed results. There could be errors in the nanodrop reading, including cross-contamination, a sample size that was too small, particulate matter in the sample, unequal volumes between tests, and more. Additionally, when the chemo drug is attached there may be errors in delivery as it is a much more complex process with the flap and binding to the receptor.

Uncontrollable Events

Our results were affected by many uncontrolled events, including shipping, our DNA passing quality control, a broken NanoDrop, and more, which delayed our project. These events created a time crunch but did not affect our project's outcome.

Conclusion

Our hypothesis was proven correct based on the results shown above. The average concentration left in solution of the modified DNA aptamer was 54.17 μM while the average concentration left in solution of the original DNA aptamer was 146.6 μM. This means that more of the modified DNA aptamer has bound to the protein and not been filtered through into the collected solution [2,15].

Practical Applications

This project is a precursor to curing cancer with high specificity and efficiency without killing as many healthy cells as possible. Aptamer AS1411- N12 is responsible for selectively binding to overexpressed proteins in cancer cells on the outer membrane [8]. AS1411- N12 serves as a biological ligand and binds to receptors with extreme levels of specificity as visualized with our results. The development of this new DNA aptamer, can improve cancer therapy and help many who are currently struggling [2,6].

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