STRUCTURE ACTIVITY RELATIONSHIP STUDIES FOR APTAMER, AFR-CM-65
Afito Djokoto¹, Prabodhika Mallikaratchy²
¹Department of Chemistry, Bronx Community College, ²Department of Chemistry, Lehman College, Bronx, New York, NY 10468

Abstract

The development of effective targeted therapies has become increasingly imperative in the treatment of cancer. Antibodies have been used for this purpose due to their precision in specificity and affinity against desired substrates. But their use can result in new toxicities and unwanted immune reactions. Recently, researchers found aptamers to be a versatile alternative. Aptamers are single or double stranded oligonucleotides that can bind to a target molecule with high affinity and specificity. They are developed by a special method called SELEX (systematic evolution of ligands by exponential enrichment). Aptamers are analogous to antibodies in terms of target recognition and applications. Aptamers have several key advantages. Aptamers have low molecular weight, low toxicities and no animal is involved in their production. They have fast tissue penetration and can be produced using a DNA synthesizer. Also, aptamers remain stable during long-term storage and can easily be labeled with fluorescence tags or radio-isotopes. In addition, they sustain reversible denaturation. These advantages make aptamers very appropriate for target cancer diagnostics and therapies.

In this work, we redesigned a version, A2, from aptamer AFR-CM-65. The aptamer AFR-CM-65 was selected against Burkitt’s lymphoma and can specifically recognize Burkitt’s lymphoma cell with high affinity.

Background

What is an aptamer?
Aptamers are single or double stranded DNA or RNA molecules that can bind to a target molecule with high affinity and specificity.

Nature of the aptamer interaction
- van der Waals Surface contacts
- Hydrogen bonds
- Stacking interactions
- Other non covalent bonds

Advantages of an aptamer
- Easy, fast and reproducible synthesis
- Easy chemical manipulation for signaling
- Fast tissue penetration
- No immune response
- Non-toxic
- Easy storage
- No animals involved in production

RNA aptamer binding to its target molecule

Goal
Systematic truncation from (3’ and 5’ end) to design shorter analogues of AFR-CM-65, to increase the thermodynamically most favorable fold.

Methods

DNA synthesis, purification and quantification

- Synthesis of DNA using solid-phase synthesis using a DNA synthesizer
- DNA de-protection
- DNA purification using reversed phase column and de-tritylation
- Spectrometric analysis and calculation of concentration

Cell-binding assay

- Incubate in ice
- Wash with a binding buffer
- Flow cytometry

Results

UV Visible absorption spectra of aptamer analogues

<table>
<thead>
<tr>
<th>Name of the sequence</th>
<th>Concentration mol/L</th>
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<tbody>
<tr>
<td>AFR-1 (76mer)</td>
<td>1.21 × 10⁻⁵</td>
</tr>
<tr>
<td>AFR-2 (55mer)</td>
<td>1.89 × 10⁻⁵</td>
</tr>
<tr>
<td>AFR-3 (64mer)</td>
<td>9.43 × 10⁻⁶</td>
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UV/Visible absorbance spectra of AFR1, AFR2 and AFR3. Absorbance maxima at 260nm was used to calculate the concentration of each molecule.

Analysis of \( P_{max} \) for AFR-CM-65, A2 and Random sequence

Concentrations from 500 nM to 12.5 nM of Random, AFR-CM-65 and A2 were incubated with 2 × 10⁵ of Ramos cells 40 minutes on ice. After washing with 3 mL, cells were reconstituted in 0.5 mL of wash buffer; binding of each molecule at each concentration was subsequently analyzed by flowcytometry. Median fluorescence of each histogram was plotted as a function of concentration.

Analysis of specificity of AFR-CM-65 and A2

(A and B) HL 60, a myeloid leukemia cells and (B and C) Daudi, a Burkitt’s lymphoma cells. A 500 nM of aptamer or random sequence was incubated with 2 × 10⁵ cells for 40 minutes in ice. Then washed the unbound sequences with 3 mL of wash buffer. Cells were suspended in 0.5 mL of wash buffer prior to flowcytometric analysis. Higher fluorescence shift on x-axis against Daudi cells compared to non-targeting myeloid cells shows that specificity of AFR is retained.

Conclusion

We have re-designed a novel analogue of AFR-CM-65 with high affinity and specificity using a simple systematic truncation approach. Future work will be aimed at stabilizing the secondary structure using unnatural DNA bases to increase the stability in human serum and at designing bivalent aptamer analogues to further increase the affinity.

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References