Targeting Telomeric and c-MYC G4 DNA as an Anticancer Approach

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Introduction

G-quadruplex (G4) DNA are non-canonical higher order DNA structures formed from guanine rich sequences, made up of stacked G-tetrads stabilized by non-Watson-Crick (Hoogsteen) base pairing and K^+ ions.

Early interests in G4 DNA were spurred on by the revelation that G4 was formed in telomeric DNA sequences at the end of our chromosomes. This was particularly promising given that G4 structures formed in telomeric DNA were also found to inhibit an enzyme known as telomerase, which is overexpressed (>90%) in cancer cells. Cancer cells require telomerase activity for survival and "immortality", therefore stabilization of telomeric G4 can inhibit telomerase activity and prevent the survival of cancer cells. More recently, G4 DNA has also been shown to be overrepresented in the promoter regions of oncogenes (e.g., c-MYC and ras genes) and the 5'UTR of mRNA. As a result, G4 DNA represents a viable target for possible anti-cancer therapeutic agents to treat previously "undruggable" sites such as the c-MYC and ras oncogenes[1].

In this work, G4 structures formed at both human telomeric and c-MYC sequences will be investigated by targeting/probing them using a variety of known and novel compounds. Using biophysical techniques such as fluorescent displacements assays, thermal melting, and circular dichroism (CD) spectroscopy, the binding characteristics of these compounds to G4 DNA will be investigated for their efficacy as a possible anti-cancer therapeutic strategy.

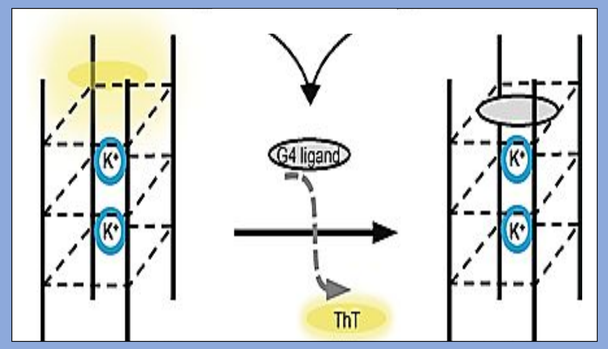

Figure 1. A simple diagram demonstrating the mechanism of the Thioflavin-T displacement assay, Adapted from Jamroskovic et al[2]

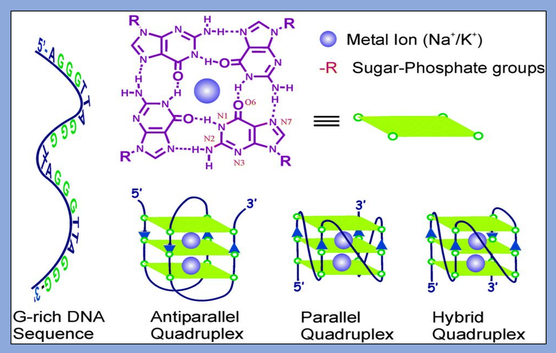

Figure 2. A simplified diagram of DNA G-quadruplexes formed from human telomeric DNA[3] .

Experimental

Sample Preparation

DNA G-Quadruplexes were formed in solution using a 10 mM phosphate buffer, pH 7, with 100mM K^+ added. Characteristic CD spectra confirmed the presence of quadruplexes in solution.

Thioflavin-T Fluorescence Displacement

Fluorescent studies were performed using a PTI Quantamaster™ 40 UV-Vis Spectrofluorometer from 400-600nm. A Fluorogenic dye that binds specifically to DNA G-quadruplexes, Thioflavin-T (Tht), was used as a fluorescent indicator for the quadruplexes. A solution of Tht-bound G-quadruplexes was prepared and the drug of interest was added. The solution was excited at 425 nm and fluorescence intensity was measured.

Circular Dichroism

Circular dichroism was performed using a Jasco-815 spectropolarimeter on myc-DNA, and myc-buffer from ranges of 200-400 nm.

Thermal Melting

Thermal melting was conducted using Varian Cary 5000 UV-vis spectrophotometer from a range of 25-95 degrees celsius. The samples assessed included: c-MYC only, c-MYC with ThT, c-MYC with the porphyrin compound TMPYP4, C-MYC with a disubstituted naphthalene diimide (NDI7).

Results

Validation of G4 Structure

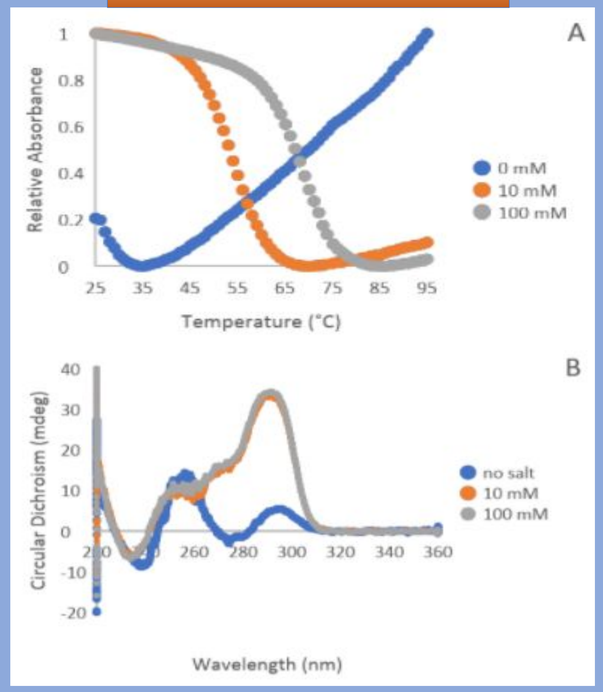

Figure 3. Thermal melting of G4 structure with varying level of ion concentration (left). Circular dichroism with varying level of ion concentration (right).

Thioflavin Displacement of NDI7 and TMPYP4

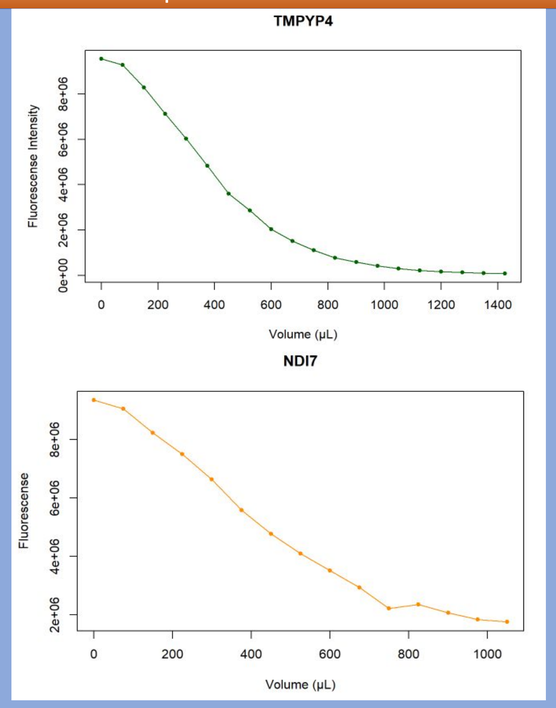

Figure 4. Thioflavin-T fluorescence displacement assays of G4 DNA with TMPYP4 and NDI7.

CD Spectroscopy of c-MYC DNA

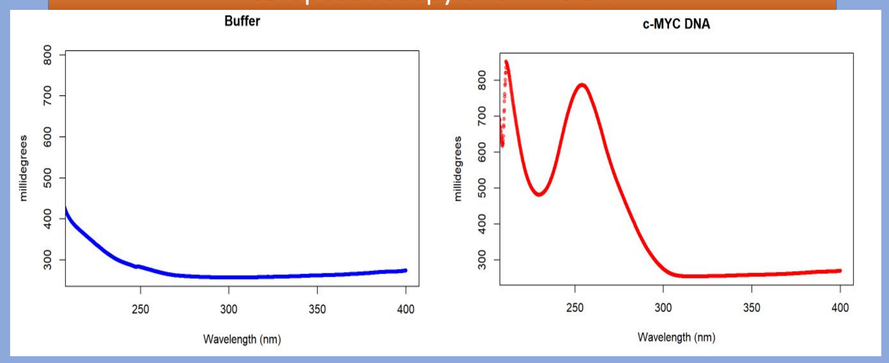

Figure 5. Circular Dichroism spectroscopy of c-MYC DNA compared to a buffer

Thermal Melting of c-MYC DNA

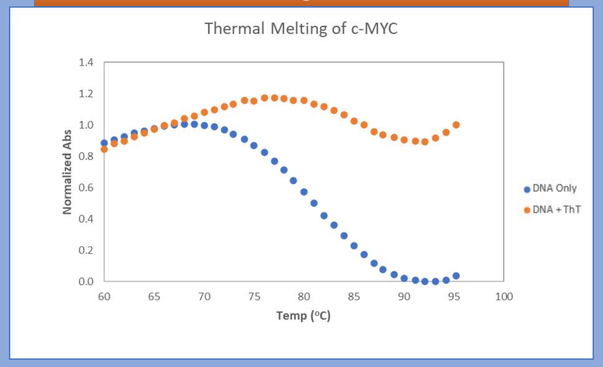

Figure 6. Thermal melting spectroscopy of c-MYC DNA and c-MYC DNA with ThT

Conclusions

- CD Spectroscopy and Thermal Melting data from previous research students confirmed the formation of telomeric G4 DNA and an ion concentration of 100mM was the most effective concentration to form these structures
- ThT displacement assays demonstrated both TMPYP4 and NDI7 bind to G4 successfully and displace ThT. Between TMPYP4 and NDI7, TMPYP4 has a higher binding affinity
- CD Spectroscopy comparing buffer and c-MYC DNA showed the successful creation of c-MYC G4 structures
- Thermal Melting confirmed the presence of c-MYC G4 structures, and ThT has the ability to bind to and stabilize these structures

Future Research

- Now that we have confirmed we created G4 c-MYC DNA, we plan to investigate the interaction of other drugs (currently in our lab, eg., additional NDI derivatives, quinacrine) with this DNA and determine whether these drugs stabilize the G4 structure
- If we can determine a drug that can target and stabilize the G4 c-MYC DNA, we may be able to use it as an anticancer approach

References

1. Brooks et al. Targeting MYC Expression through G-Quadruplexes. SAGE. 2010; 10.1177/19476010377493
2. Jamroskovic et al. Identification of Compounds that Selectively Stabilize Specific G-Quadruplex Structures by using a Thioflavin T-Displacement Assay as a tool. Chemistry – A European Journal. 2016; 52:18932-18943
3. Mohanty et al. Thioflavin T as an Efficient Inducer and Selective Fluorescent Sensor for the Human Telomeric G-Quadruplex DNA. Journal of the American Chemical Society. 2013; 135:367-376

Acknowledgments

We acknowledge the Geneseo Foundation and the Department of Chemistry for the instruments used to collect our data and funding to acquire the c-MYC DNA, Dr. Kazushige Yokoyama at SUNY Geneseo for the Thioflavin-T used in our displacement studies, Dr. Tracy Brooks at SUNY Binghamton for her experimental input, and Medhi Elmouchtari, Joseph Kanlong, and Sarah Ostrowski Geneseo alums who began Dr. McKnight's work on G-quadruplexes.