UNDERSTANDING THE STRUCTURE AND STABILITY OF QUADRUPLEX NUCLEIC ACIDS FORMED FROM HUMAN TELOMERIC SEQUENCES

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BY

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To the Dean of the Graduate School:

I am submitting herewith a thesis written by Brenna Alyssa Tucker entitled "Understanding the Structure and Stability of Quadruplex Nucleic Acids Formed from Human Telomeric Sequences." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science with a major in Chemistry.

Dr. Richard Sheardy, Major Professor

We have read this thesis and recommend its acceptance:

Department Chair

Accepted:

Dean of the Graduate School

DEDICATION

To my dear sweet grandparents, Mr. and Mrs. Roy and Selita Tucker, thank you for always having faith in me. I am so honored to have made you so proud.

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ABSTRACT

BRENNA ALYSSA TUCKER

UNDERSTANDING THE STRUCTURE AND STABILITY OF QUADRUPLEX NUCLEIC ACIDS FORMED FROM HUMAN TELOMERIC SEQUENCES

AUGUST 2011

The human telomere is a sequence of DNA found at the ends of chromosomes consisting of the repeat d(TTAGGG) and serves to protect genetic information against chromosomal degradation. The human telomere is unique in its ability to fold into a G-quadruplex secondary structure with the ability to inhibit telomerase, increasing the interest of G-quadruplex structures over the years. Our work with the human telomere is based upon three main questions: How is the structure and stability of quadruplexes affected by 1) altering the sequence of the loops?; 2) modifying the length of the repeat?; and 3) changing the nature of the counter ion present in solution? In order to answer these questions we have studied all permutations of $(XXXGGG)_4$ where X= T or A, $(TTAGGG)_x$ where x= 1, 2, or 4, as well as investigate the effects of sodium and/or potassium upon all sequences in our library.

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CHAPTER I

INTRODUCTION

Quadruplex DNA Background

Deoxyribonucleic acid (DNA) is most commonly pictured as the classic DNA double helix seen in Figure 1.1A ("DNA Bases"). In this model solved by James D. Watson and Francis Crick in 1953, two individual strands of DNA interact to form base pairs between the guanine (G) and cytosine (C) bases as well as the thymine (T) and adenine (A) bases (737-38). Known as Watson-Crick base pairing, G and C bases are paired together by three hydrogen bonds while T and A bases are paired together by only two hydrogen bonds. The differences in hydrogen bonding between these base pairs leads to differences in the stability (i.e. melting temperature) of sequences depending upon the ratio of G-C to T-A base pairs.

Since 1953, several other DNA structures with different molecularities and conformations have been observed. A single strand of self-complementary DNA folded over and base paired to itself is known as a DNA hairpin (Figure 1.1B). These structures form through Watson-Crick base pairing and can be identified by their unpaired loop region and base paired stem region ("Dimer with Hairpin Loop"). Triplexes can be formed when three strands of DNA associate with each other (Figure 1.1C). The third strand, usually a single stranded region of DNA, utilizes Hoogsteen (see below) bonding to align itself along the major groove of a DNA duplex (Seidman, and Glazer 487-94).

Quadruplex secondary structure is very different than other types of DNA structures. Making use of Hoogsteen base pairing, quadruplexes can be formed through the association of one, two, or four strands of DNA (Figure 1.1D).

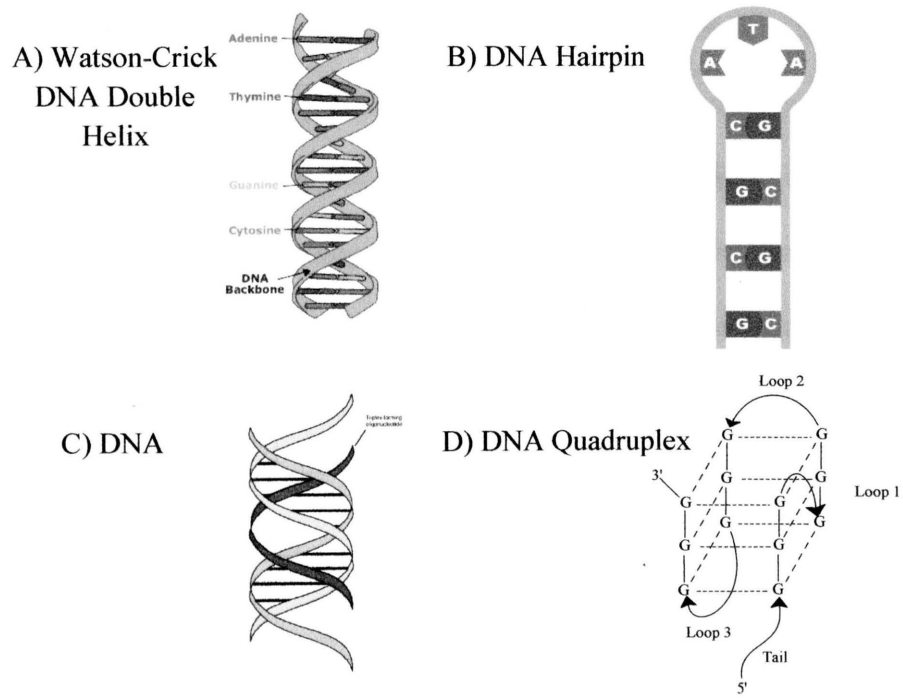

Figure 1.1: Selected DNA secondary structures. DNA is highly polymorphic and can form a variety of secondary structures. Here four different structures are shown.

Quadruplexes can be formed from single stranded DNA or RNA provided that the sequences contain contiguous tracts of two - four guanine bases. They are characterized by the formation of Guanine (G)-tetrads (Figure 1.2), or four guanine bases oriented through Hoogsteen base pairing with a square co-planar geometry (Antonacci, Chaires, and Sheardy 4654-60). The Hoogsteen base pairing occurs between the N1 and N2 of one

G base with the O6 and N7 of the neighboring G base. This arrangement allows for the creation of eight hydrogen bonds within each tetrad increasing the stability of these structures.

The G-tetrads stack upon one another in a right handed helical fashion in order to form the quadruplex, creating a central pore throughout the length of the structure. Monovalent metal cations like potassium or sodium are situated in the central pore in between the G-tetrad layers and help to stabilize the structure (Balagurumoorthy et al. 4061-67). Different folding topologies are observed when quadruplexes are formed in the presence of different cations, a trait most likely due to size difference of ionic radii. Although divalent cations like Mg^{2+}, Ca^{2+} and Sr^{2+} can induce quadruplex formation, they do not stabilize the structure to the extent of the monovalent cations (Dai, Marotta, and Sheardy 3655-62; Venczel, and Sen 6220-28).

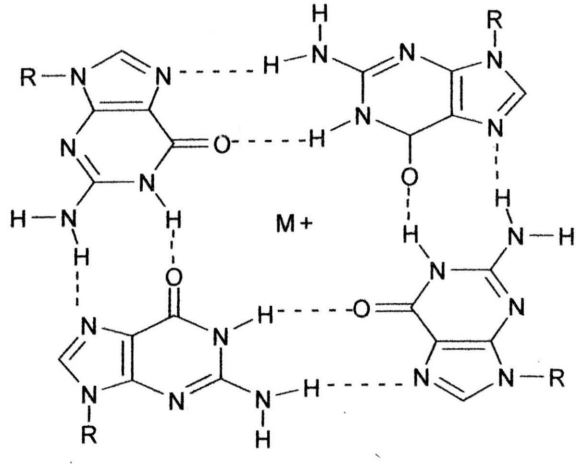

Figure 1.2: The guanine tetrad. A characteristic of quadruplex DNA, the G-tetrad forms through the Hoogsteen base pairing of 4 G bases arranged in a square co-planar fashion.

Quadruplex forming oligomers of DNA have been shown to exhibit different molecularties of one, two, or four depending upon the length and the environment of the individual strands (Balagurumoorthy et al. 4061-67; Williamson 703-30; Parkinson, Lee, and Neidle 876-80; Balagurumoorthy, and Brachmachari 21858-69; Ambrus et al. 2723-35). Depending upon the molecularity of the quadruplex, the structures can be influenced to exhibit different strand orientations of parallel, anti-parallel, or a combination of both (Olsen, Gmeiner, and Marky 6962-69; Wang, and Patel 263-82). Figure 1.3 depicts the different molecularities and strand orientations that quadruplex structures can fold into. The G bases of the tetrads have an anti conformation when the strand orientation is all parallel but alter between syn-anti-syn when any strand runs anti-parallel to the others.

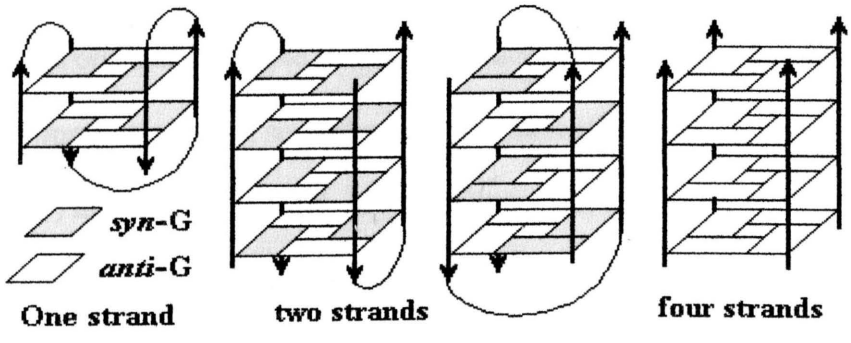

Figure 1.3: Molecularities of quadruplexes. The number of strands that associate to form a quadruplex determines the molecularity. Here molecularities of 1, 2, and 4 are shown. Arrow heads indicate the strand orientation.

The molecularity and strand orientation of quadruplexes are also dependent upon the loop regions of the structure. The sequence, as well as the length, of the loop regions influences how the structure is folded into a quadruplex (Risitano, and Fox 2598-606). Several different loops have been classified including: lateral loops, diagonal loops, and strand reversal loops. Loops that are shorter than three bases do not have the ability to form diagonal loops and can only form lateral or strand reversal loops (Parkinson, Lee, and Neidle 876-80). In an intramolecular quadruplex, strand reversal loops allow for an all parallel strand orientation with at least one neighboring strand whereas the other two types of loops lead to an anti parallel strand orientation with at least one neighboring strand.

Quadruplex forming sequences can be found in many different DNA sequences within eukaryotic cells. The sequence known as the human telomere, found capping chromosomes, is one such example (Chan, and Blackburn 109-21). Other examples include the promoter regions of several oncogenes including c-myc (Gonzalez, and Hurley 9706-14) and Bcl-2 (Dai et al. 1096-98) as well as the gene for VEGF (vascular endothelial growth factor) (Guo et al. 4598-608).

Telomeres and Telomerase

Telomeres are important sequences of non-coding DNA that serve as an end-cap to chromosomes in order to prevent them from chromosomal degradation. The human telomere is located at the end of chromosomes and consists of approximately 30,000 base pairs repeating the sequence d(TTAGGG). The last 100 to 200 bases exist as a single

stranded 3' overhang and plays an important role in telomere structure and function (Chan, and Blackburn 109-21; Moyzis et al. 6622-26). The single stranded region of the human telomere has the ability to fold into multiple quadruplexes because of the many continuous tracts of three G nucleotides.

Oligomers of the human telomere have been shown to fold into a quadruplex exhibiting both parallel and strand reversal loops when in the presence of K^+ (Dai, Carver, and Yang 1172-83). Of the three loops, the first is a strand reversal loop and the second two are parallel loops (Figure 1.1D). The strand reversal loop allows the structure to have a combination parallel anti-parallel strand orientation.

Sequences that have the ability to form quadruplexes have been studied over the past several decades because of their association with anti-aging and anti-cancer (Neidle 1118-25; Neidle, and Parkinson 383-93). One of the most important potential functions for telomeric quadruplex structures is its ability to inhibit telomerase, a reverse transcriptase enzyme which works in some cells to maintain the length of the telomere (Chan, and Blackburn 109-21). When normal cells divide and replicate, short stretches of the telomere are removed, roughly, anywhere between 20 and 200 base per replication cycle. After several replication cycles, the telomere becomes so short that the cell is no longer able to replicate and undergoes apoptosis, or programmed cell death. Instead of the telomere shortening over time in cells that contain telomerase, the length of the telomere is maintained and the cell never reaches apoptosis.

Approximately 85-90% of cancerous cells contain the enzyme telomerase (Coviello et al. 2011). Because quadruplexes have the ability to inhibit telomerase, there has been an increase in the amount of attention that these structures have been receiving as a potential target for anti-cancer therapy. It is very important that we have a firm understanding of the different structures that they can form as well as their respective stabilities in order to use quadruplexes in developing new cancer therapeutics.

To learn more about how the quadruplex structure is able to inhibit telomerase, we have developed three different approaches to study the structures and corresponding stabilities of many different quadruplex forming sequences. Our first approach, or specific aim, is to determine the effect of modifying the bases in the loops of the quadruplex structure. Our second specific aim is to determine the relationship between the number of times the TTAGGG sequence is repeated and the structure and stability of the resulting quadruplex. The third specific aim seeks to identify what role the nature of the counter ion plays upon the structure and stability of the quadruplex.

Spectroscopic Theory

Several different spectroscopic methods can be employed in order to derive information about the structures and corresponding stabilities of quadruplex forming sequences. In this work we use a combination of UV/Vis spectroscopy as well as circular dichroism spectroscopy in order to determine the concentration of DNA in solution and for estimation of DNA secondary structure, respectively.

DNA absorbs light between the range of 240-280 nm with a maximum absorption around 260 nm. DNA absorption can be attributed to the purine and pyrimidine bases of the structure. We can be sure of this because the ribose-phosphate backbone of the DNA does not affect the absorption in this particular range. The wavelength of maximum absorbance (λ_{max}) and the extinction coefficient (ε) at λ_{max} is dependent upon several different factors including the base composition, the base-pairing interactions present, the salt concentration of the solution, and the pH of the solution.

Because DNA is able to absorb light, we can use Beer's Law to determine the concentration of the DNA in a given sample. Beer's Law states that:

$$A = \varepsilon \ell c \qquad \text{(Equation 1.1)}$$

where A = absorbance, ε = the molar extinction coefficient, ℓ = the pathlength of the cuvette, and c = molar concentration. If the molar extinction coefficient of a sample is known, then the concentration can easily be determined by obtaining and substituting the A_{260} value, the extinction coefficient and the path length of the cuvette into Beer's Law and solving for the concentration value.

DNA optical melting refers to monitoring the change in absorbance of the sample at a particular wavelength while there is an increase in temperature. Upon an increase in temperature, the DNA structure unfolds into a single strand. Upon cooling in reversible processes, DNA can anneal or re-fold into its secondary structure. Some annealing

processes can take longer than others depending upon the length of the sequence and other environmental effects such as pH and salt concentrations.

When the A_{260} values are plotted against the temperature, a sigmoidal curve is obtained that shows the cooperative transition between the folded and unfolded structures. The sigmoidal shape is due to the differences in molar extinction coefficients of double stranded and single stranded DNA. As we increase the temperature, the strand begins to denature causing the molar extinction coefficient to increase. The midpoint of the transition on the curve is directly related to the transition midpoint, or the melting temperature of the sample.

We have to keep in mind that this analysis assumes a two-state transition, meaning that if the sample goes from completely folded to completely unfolded without forming any other structures, we can use this model to determine the melting temperature and calculate the corresponding enthalpy, entropy and free energy of the transition. If the transition is not two-state, than other techniques must be used to obtain the melting temperature.

Circular Dichroism (CD) is a spectroscopic technique that measures the difference in absorption of left and right circularly polarized light. When circularly polarized light hits a chiral sample, the right and left hand components of the light are absorbed differently. The difference between the left hand absorption and the right hand absorption is a phenomenon known as ellipticity:

$$\Theta = \Theta_l - \Theta_r \qquad \text{(Equation 1.2)}$$

where Θ = ellipticity, Θ_l = left handed absorption, and Θ_r = right handed absorption. For a CD signal to be observed, the molecule under study must be chiral due to lack of the symmetry and must be chromophoric.

Circular Dichroism can be used to determine types of secondary structures of nucleic acids, like a duplex or a quadruplex as well as proteins, such as alpha helices and beta sheets. CD is also a technique commonly used as a tool for studying nucleic acid – ligand interactions (Paramasivian, Rujan, and Bolton 324-31). Information can only be inferred from CD spectra, but when coupled with other techniques such as differential scanning calorimetry, there is much we can learn about the sample.

Circular Dichroism spectra are most often presented as a plot of molar ellipticity versus wavelength. Molar ellipticity is a preferred unit of measurement because the concentration of the sample is considered, allowing for the comparison of different data. The relationship between observed ellipticity and molar ellipticity is illustrated by the following equation:

$$[\Theta] = \theta/c\ell \qquad \text{(Equation 1.3)}$$

where $[\Theta]$ = molar ellipticity, Θ = ellipticity, c = the concentration of the sample, and ℓ = the path length of the cuvette.

CHAPTER II

MATERIALS AND METHODS

Preparation of Buffers

Potassium standard buffers (150 mM K^+, 10 mM PO_4^{-3}, and 0.1 mM EDTA) at pH 7.0 were prepared by dissolving 0.680 g KH_2PO_4 (VWR International Lot # 46032627), 0.870 g K_2HPO_4 (VWR International Lot # 46205641), 10.1 g KCl (VWR International Lot # 46100627), and 0.037 g EDTA (EMD Chemicals Lot # 45166714) in 1 L deionized water at room temperature. 10X potassium buffers (150 mM K^+, 100 mM PO_4^{-3}, and 1.0 mM EDTA) were also prepared by combining 6.80 g KH_2PO_4, 8.71 g K_2HPO_4, and 0.372 g EDTA in 1 L deionized water. All buffers were filtered through a 0.45 μm Millipore filter before being stored for use.

Sodium standard buffers (150 mM Na^+, 10 mM PO_4^{-3}, and 0.1 mM EDTA) at pH 7.0 were prepared by dissolving 0.690 g NaH_2PO_4 (GFS Chemicals Lot # P454918), 0.710 g Na_2HPO_4 (Fisher Chemicals Lot # 000363), 7.89 g NaCl (VWR International Lot # 47253743), and 0.037 g EDTA in 1 L deionized water at room temperature. 10X sodium buffers (150 mM Na^+, 100 mM PO_4^{-3}, and 1.0 mM EDTA) were also prepared by combining 6.90 g NaH_2PO_4, 7.10 g Na_2HPO_4, and 0.372 g EDTA in 1 L deionized water. All buffers were filtered through a 0.45 μm Millipore filter before being stored for use.

Titration buffers at pH 7.0 were prepared by combining the appropriate volume of the 10X potassium and sodium buffers along with 0.5 M KCl (18.6 g KCl in 500 mL deionized water) and 0.5 M NaCl (14.6 g NaCl in 500 mL deionized water).

Preparation of Sequences

All HPLC-purified oligomers were purchased from Bio-synthesis, Inc (Lewisville, TX) and used without further purification. Each sequence was prepared by reconstituting 1 μmole of each oligomer in 1 mL of the standard phosphate buffer. Each sample was heated to and held at 95 °C for 5 minutes before slowly cooling to room temperature. After sitting at room temperature for several hours, the samples were incubated at 5 °C for 24 hours before use in order to ensure proper annealing.

Samples used in titrations were prepared by dissolving 1 μmole of $(TTAGGG)_4$ in 1 mL of deionized water and splitting the contents among 11 eppendorf tubes. The water was evaporated off using a Savant DNA 110 Speed Vac and 1 mL of the appropriate titration buffer was added to each sample. The samples were then heated to and held at 95 °C for 5 minutes before slowly cooling to room temperature. After sitting at room temperature for several hours, the samples were incubated at 5 °C for 24 hours before use.

UV/Vis Spectroscopy

UV/Vis studies to determine concentration were performed with a Varian Cary 100 Bio model (Varian Associates, Palo Alto, CA). Appropriate dilutions of each sample were made and run from 320 – 220 nm at 25 and 95 °C with the buffer baseline

subtracted. Samples were run in a 5 mm square quartz cuvette. Concentrations were calculated at 25 °C using Beer's Law and the molar extinction coefficient at 260 nm for each oligomer as provided by the manufacturer (see Table 2.1).

Circular Dichroism

All CD studies were performed using an Olis RSM 1000 spectrophotometer (Olis, Inc. Athens, GA). Data was collected over the range of 320 – 220 nm at both 25 and 95 °C with an integration time of 3 seconds. A baseline of the empty sample chamber was obtained from 320 – 220 nm using an integration time of 0.5 seconds was subtracted from each spectrum. Samples were run in a 1 mm circular quartz cuvette. Data was analyzed using Olis Global Works and SigmaPlot version 11.

Table 2.1:

Molar Extinction Coefficients for Quadruplex Forming Oligomers.

Loop Sequence Effects		*Number of Repeats Effects*	
Oligomer	ε (mol^{-1} bases cm^{-1})	Oligomer	ε (mol^{-1} bases cm^{-1})
$(TTAGGG)_4$	243717.6	$(TTAGGG)_1$	60929.4
$(TATGGG)_4$	243717.6	$(TTAGGG)_2$	121858.8
$(ATTGGG)_4$	243717.6	$(TTAGGG)_4$	243717.6
$(TTTGGG)_4$	220114.8	$(TTAGGG)_8$	487435.2
$(AATGGG)_4$	267320.4		
$(ATAGGG)_4$	267320.4		
$(TAAGGG)_4$	267320.4		
$(AAAGGG)_4$	290923.2		

CHAPTER III

RESULTS AND DISCUSSION

Loop Sequence Context

Our first specific aim is to identify the effect of modifying the loop sequence context of $(TTAGGG)_4$ on the structure and stability of each of the resultant quadruplexes. In order to do this we have considered all permutations of $(XXXGGG)_4$, where X= T or A as seen in Table 3.1.

Table 3.1:

Oligomers Used for Loop Sequence Context Studies

Name	Sequence
HTEL 1	TTAGGGTTAGGGTTAGGGTTAGGG
HTEL 1A	TATGGGTATGGGTATGGGTATGGG
HTEL 1B	ATTGGGATTGGGATTGGGATTGGG
HTEL 1C	TTTGGGTTTGGGTTTGGGTTTGGG
HTEL 1D	AATGGGAATGGGAATGGGAATGGG
HTEL 1E	AAAGGGAAAGGGAAAGGGAAAGGG
HTEL 1F	ATAGGGATAGGGATAGGGATAGGG
HTEL 1G	TAAGGGTAAGGGTAAGGGTAAGGG

Quadruplexes have signature CD spectra and can be identified by a peak at either 265 or 295 nm and a trough at 240 nm or 265 nm, respectively (Balagurumoorthy, and Brachmachari 21858-69). Studies on these sequences in 150 mM K^+ show that each gives the characteristic CD spectrum for a quadruplex even though the CD spectra for each sequence differs from the next as can be seen in Figure 3.1. Figure 3.1A depicts the CD

spectra of sequences which contain 2 or more T bases while Figure 3.1B shows the CD spectra of sequences that contain 2 or more A bases. From the differences in the CD spectra, we can conclude that the loop sequence context directly influences the folding topology of the resulting quadruplex.

The CD spectra for these sequences have also been collected over a range of temperatures in order to establish an optical melting profile and obtain a melting temperature (T_m) for each of the strands. The molar ellipticity at a particular wavelength plotted against temperature gives us sigmoidal curves that we can use to derive T_m values from (Figure 3.2). From this plot we can see that when the loops are replaced with all A bases, the stability in relation to melting temperature has drastically decreased. Interestingly enough, the human telomeric oligomer does not have the highest T_m as one might expect, it has the third highest T_m. A summary of the T_m values for each oligomer can be found in Table 3.2.

Singular Value Decomposition (SVD) of this data shows that three significant spectral species are present during the unfolding of each of the quadruplexes, meaning that they unfold via a three state transition. These findings however, cause questions to arise about the validity of the T_m values obtained from the optical melting profiles which assume a two state transition. The T_m values established through the optical melts can only be used to determine a relative stability between the strands. In order to properly characterize these oligomers based upon their stability in regards to T_m values, they must be examined through calorimetric techniques.

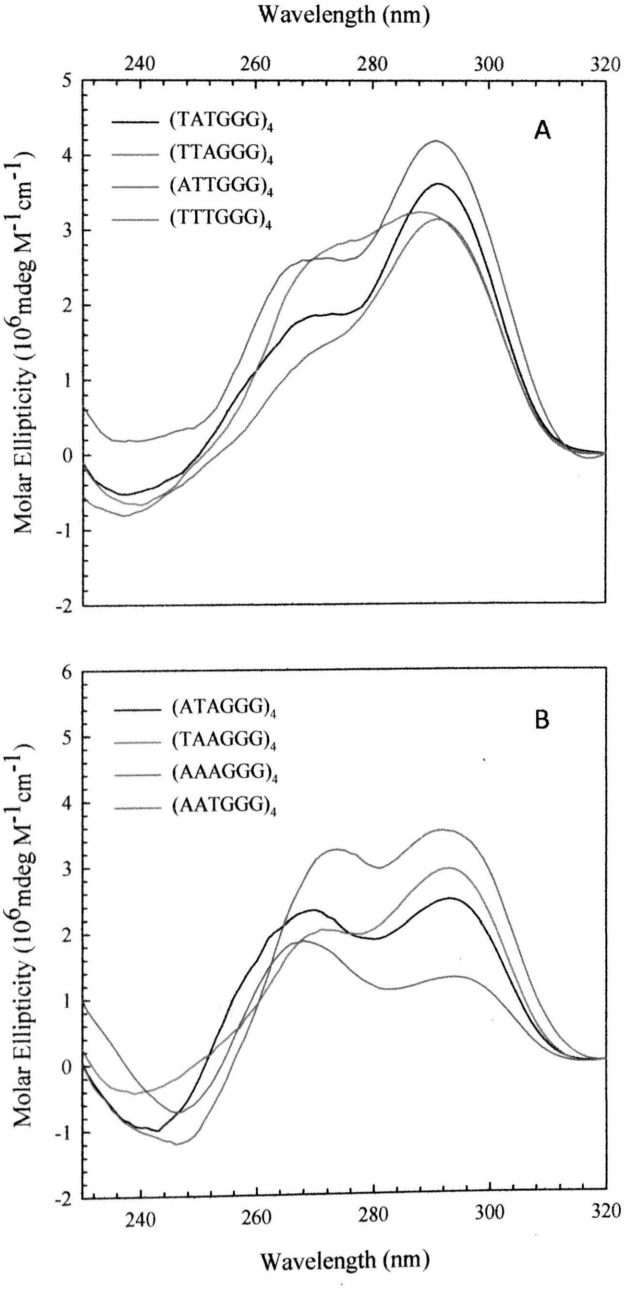

Figure 3.1: CD spectra of the $(XXXGGG)_4$ oligomers. Panel A depicts sequences with 2 or more T bases and Panal B depicts sequences with 2 or more A bases. Spectra were obtained in 10 mM phosphate buffer, pH 7.0 and 150 mM K^+ at 25 °C.

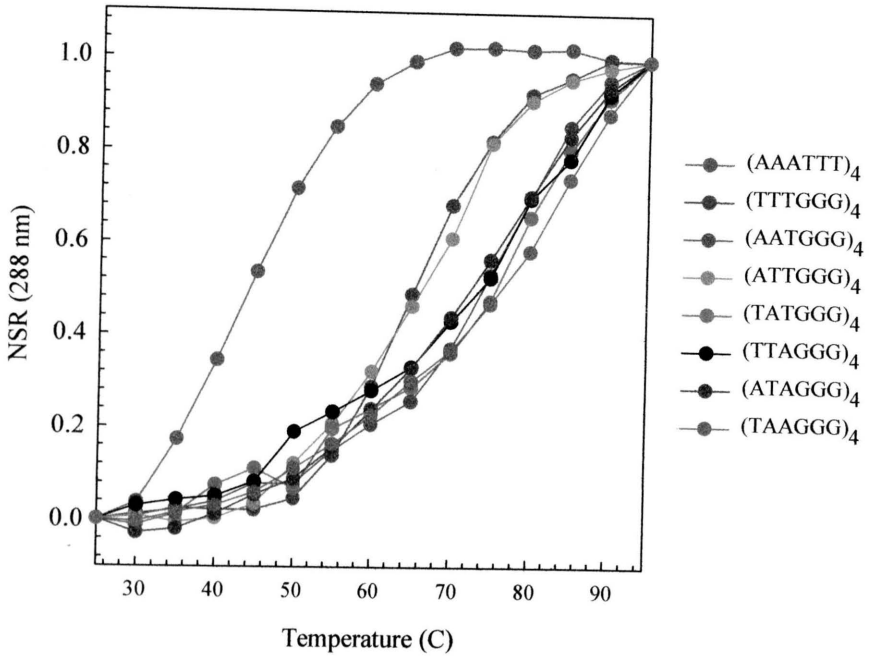

Figure 3.2: CD optical melting profiles for the $(XXXGGG)_4$ oligomers. The NSR is defined as the fraction of the single strands throughout the transition and is calculated by $(\theta_{25} - \theta_T)/(\theta_{25} - \theta_{95})$ where θ_{25} is the molar ellipticity at 25 °C, $\theta_{95}$is the molar ellipticity at 95 °C and θ_T is the molar ellipticity at temperature T. The midpoint of the transition is defined as T_m.

Table 3.2:

Melting data for the $(XXXGGG)_4$ Oligomers

Oligomer	*"T_m" (°C)*	*SVD Spectral Species*
$(TTAGGG)_4$	73.5	3
$(TATGGG)_4$	75.8	3
$(AATGGG)_4$	66.5	3
$(TTTGGG)_4$	65.2	3
$(AATGGG)_4$	74.2	3
$(ATAGGG)_4$	73.9	3
$(TAAGGG)_4$	74.7	3
$(AAAGGG)_4$	44.2	3

Length Effects

To understand the complex nature of quadruplexed nucleic acids we must investigate how changing the length, or the number of times the TTAGGG sequence is repeated, influences the structure and stability of oligomers of the human telomere. We began our study by analyzing the sequences listed in Table 3.3 where TTAGGG is repeated once, twice, four, and eight times. Each of these sequences was evaluated by circular dichroism spectroscopy in either 150 mM potassium or sodium and compared to determine differences in molecularities and strand orientations.

Table 3.3:

Oligomers Used to Study Length Effects.

Name:	Sequence:
HTEL 1	TTAGGGTTAGGGTTAGGGTTAGGG
HTEL 3	TTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGG
HTEL 4	TTAGGGTTAGGG
HTEL 5	TTAGGG

Figure 3.3 shows the CD spectra of the sequences listed in Table 3.3 in the presence of 150 mM K^+. TTAGGG as well as $(TTAGGG)_2$ both have peaks around 265 nm and troughs around 240 nm, indicative of a tetra-molecular all parallel quadruplex (Dapic et al. 2097-107). $(TTAGGG)_4$ and $(TTAGGG)_8$, have peaks around 290 nm,

shoulders around 265 nm, and troughs around 240 nm. These sequences have CD spectrums that show characteristics of both all parallel structures because of their trough at 240 nm as well as anti-parallel structures, because of their peak at 290 nm (Dapic et al. 2097-107; Paramasivian, Rujan, and Bolton 324-31). Sequences that give this type of CD signal are thought to have a combination parallel, anti-parallel intramolecular quadruplex secondary structure (Dapic et al. 2097-107). From the CD spectrum of these sequences we can see that the number of times TTAGGG is repeated affects the molecularity as well as the strand orientation of the resulting quadruplex.

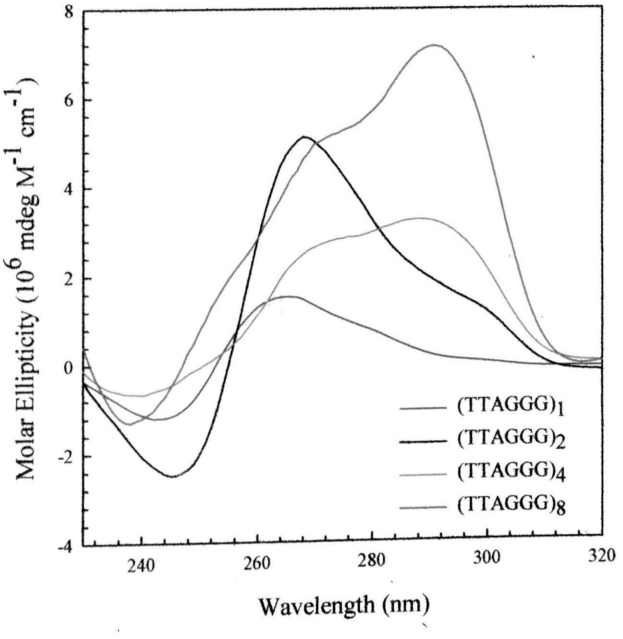

Figure 3.3: CD spectra of $(TTAGGG)_x$ sequences in 150 mM K^+. Spectra were collected in 10 mM phosphate buffer, pH 7.0 and 150 mM K^+ at 25 °C.

When the same sequences are in the presence of 150 mM Na^+, we observe differences in the behaviors of the oligomers as can be seen in Figure 3.4. TTAGGG also forms an all parallel tetramolecular quadruplex in the presence of Na^+ because it maintains its peak at 265 nm and trough around 240 nm. The differences arise when the other sequences are inspected. When TTAGGG is repeated twice, four, and eight times in the presence of Na^+ two peaks are observed with the first centered around 295 nm and the second centered around 250 nm. Two troughs are also observed for these sequences with the first located at 270 nm and the second at 240 nm. The CD spectra of these sequences indicate that they fold into chair type anti parallel quadruplex (Paramasivian, Rujan, and Bolton 324-31).

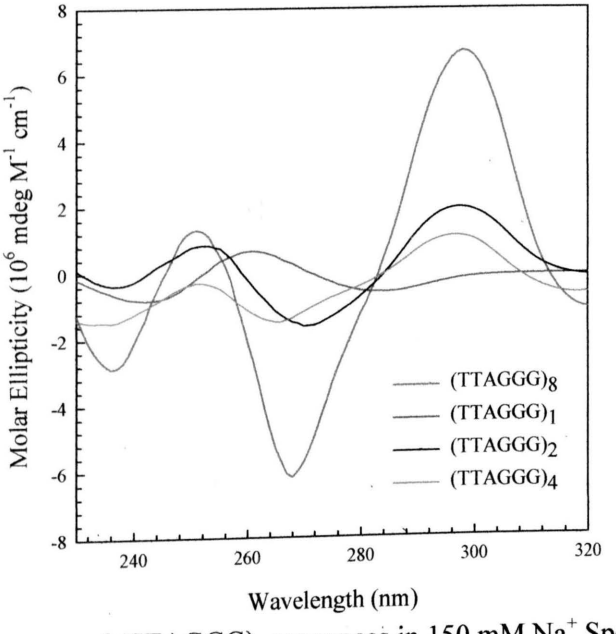

Figure 3.4: CD spectra of $(TTAGGG)_x$ sequences in 150 mM Na^+ Spectra were collected in 10 mM phosphate buffer, pH 7.0 and 150 mM Na^+ at 25 °C.

When the length of the single stranded region of the human telomere is considered, there exists a possibility that more than one quadruplex structure in a row can be formed. The formation of multiple quadruplexes in a row is an interesting concept that could lead to an increased stability of the strands. In this work we attempted to show the existence of a double quadruplex, that is two intramolecular quadruplexes in a row, through a spectroscopic comparison.

Figure 3.5 shows $(TTAGGG)_4$ and $(TTAGGG)_8$ in the presence of 150 mM K^+ along with a plot of $(TTAGGG)_4$ added to itself. The CD spectra of $(TTAGGG)_4$ added to itself has the same shape and intensity as the CD spectra of $(TTAGGG)_8$, indicating that $(TTAGGG)_8$ can form two intramolecular quadruplexes in a row. The similarities in the intensities of the bands tell us that each quadruplex formed folds with the same conformation.

Interestingly enough, when the same plot is created for sequences in the presence of sodium, the same results cannot be obtained (Figure 3.6). This is most likely due to the coordination of the K^+ and Na^+ ions between the tetrad layers. K^+ ions coordinate with all eight surrounding O6 atoms of the guanine bases while Na^+ ions do not (Parkinson 1-27). This means that the K^+ ions will be found in a singular location within the quadruplex while Na^+ ions can be found in multiple locations. These differences allow quadruplexes formed in the presence of K^+ ions to be more consistent with their shape because of the single location the K^+ can occupy within the G tetrads.

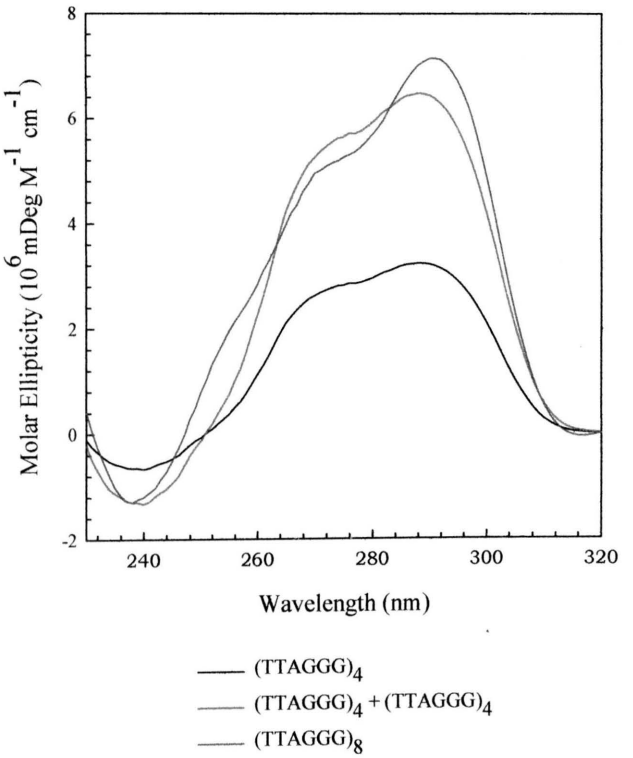

Figure 3.5: CD of single and double quadruplexes in K^+. Spectra were collected in 10 mM phosphate buffer, pH 7.0 and 150 mM Na^+ at 25 °C.

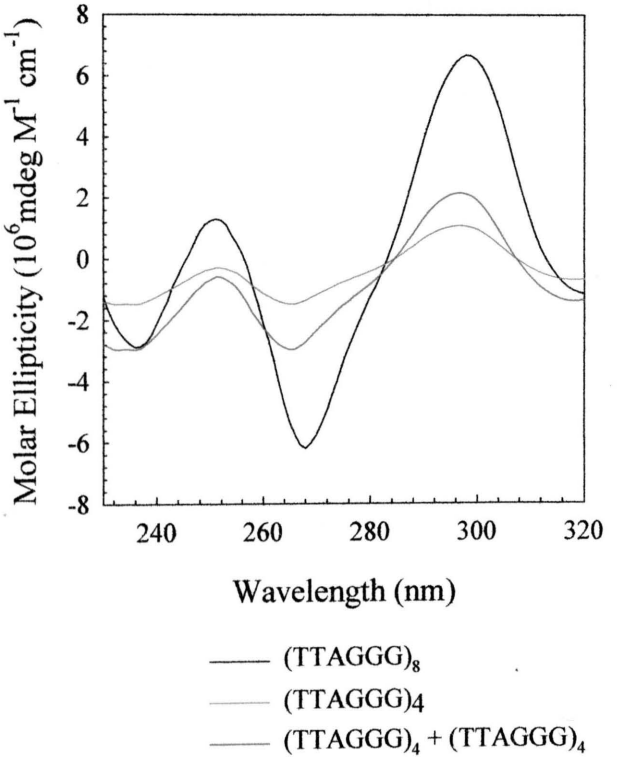

Figure 3.6: CD of single and double quadruplexes in Na^+. Spectra were collected in 10 mM phosphate buffer, pH 7.0 and 150 mM Na^+ at 25 °C.

Cation Effects

Determining how the nature of the counter ion influences the structures and stabilities of quadruplexes is crucial in characterizing human telomeric DNA sequences. Potassium and sodium are commonly used as counter ions due to their monovalent charge and biological relevance. As discussed previously, the location of the cation in crystal structures can differ depending upon the cations present in solution leading to drastic differences in secondary structure. In order to evaluate the effects of K^+ and Na^+ upon our sequences, we have spectroscopically characterized all oligomers in our library in the presence of either sodium or potassium.

Figure 3.7 shows CD spectra of $(TTAGGG)_4$ in different concentrations of either K^+ or Na^+. In this plot, we can see two distinct spectral bands that are obtained whenever a quadruplex is formed in the presence of either K^+ or Na^+. All plots of the quadruplexes formed in different concentrations of K^+ have a peak at 295 nm, a shoulder at 270 nm and a trough at 240 nm. This type of signature is characteristic of an intramolecular quadruplex with a combination parallel/ anti-parallel strand orientation.

Examining the spectra obtained from the quadruplexes formed in different concentrations of Na^+, we see similar results to the K^+ quadruplexes. The shape of the CD spectra for Na^+ quadruplexes is different from the K^+ quadruplexes, but all solutions exhibit conserved behavior just like the K^+ quadruplexes. All Na^+ quadruplexes have two peaks with the first being located at 250 nm and the second at 300 nm as well as two troughs located at 240 and 265 nm. All of these solutions exhibit CD spectra that are

characteristic of an intramolecular chair type quadruplex with an anti parallel strand orientation (Dapic et al. 2097-107; Paramasivian, Rujan, and Bolton 324-31). From Figure 3.7 we can conclude that because the CD signals of the quadruplex solutions overlay, there is no dependence of the secondary structure formation upon the concentration of either K^+ or Na^+. It is the presence of a particular counter ion that influences the folding topology of the quadruplex.

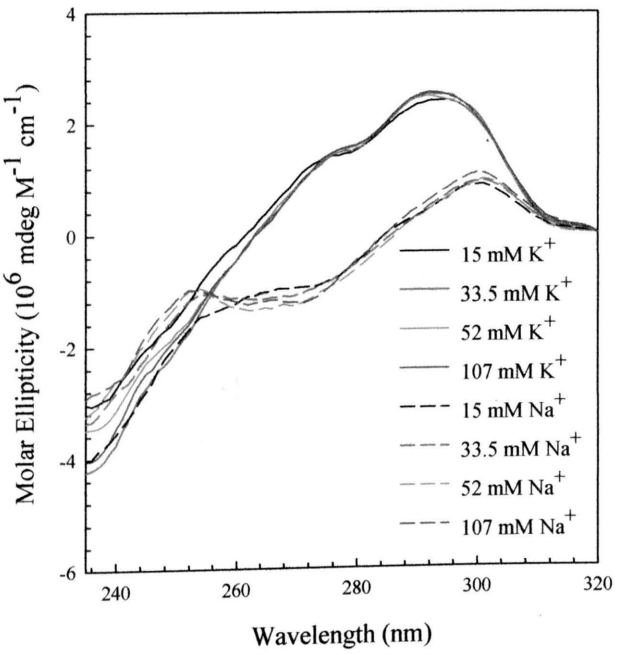

Figure 3.7: Cation dependence of $(TTAGGG)_4$. Shown are the CD spectra of $(TTAGGG)_4$ in varying concentration of K^+ or Na^+. Sequences were prepared in 10 mM phosphate buffer, pH 7.0 and the indicated amount of either K^+ or Na^+. Spectra were collected at 25 °C and strand concentrations were in the range of 1.05×10^{-5} to 1.20×10^{-5}.

Both K^+ and Na^+ ions are found in the cell at different concentrations with K^+ being found at slightly higher concentrations. Keeping this in mind, we want to explore how the combination of both K^+ and Na^+ ions will affect the structure and stability of $(TTAGGG)_4$. We were able to complete this work by analyzing $(TTAGGG)_4$ in solutions of varying ratios of K^+ to Na^+ while keeping the total ionic strength of each solution constant.

For the first titration performed a total ionic strength was maintained at 200 mM K^+ plus Na^+. Figure 3.8 shows the CD spectra of these solutions. We can see a clear transition from the Na^+ form of the quadruplex to the K^+ form of the quadruplex. When the experiment was repeated at 150 mM total ionic strength, a similar transition occurs as can be seen in Figure 3.9. From these plots we are able to construct a transition plot in reference to the change in the molar ratios of K^+:Na^+.

An overlay of both the 200 mM and 150 mM total ionic strength titration transition curves yields the same results (Figure 3.10). For both titrations the midpoint of the curve, or the midpoint of the transition from the sodium form of the quadruplex to the potassium form of the quadruplex, occurs at 0.11 mole fraction of K^+. The transition is complete by 0.45 mole fraction of K^+, that is the quadruplex requires less potassium in solution than sodium in order to for the potassium form of the quadruplex.

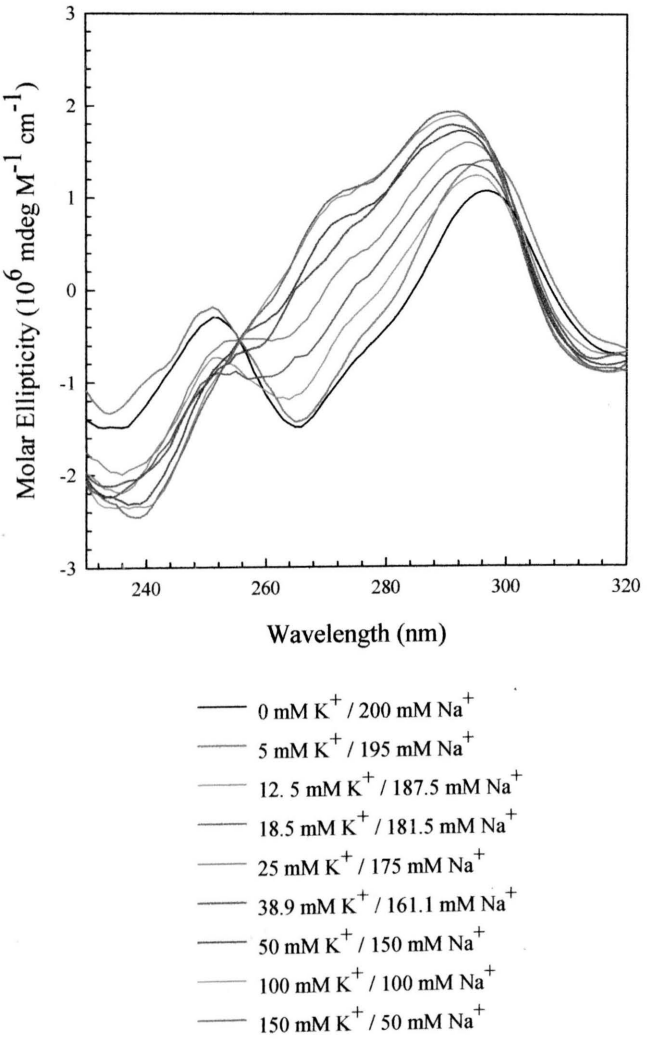

Figure 3.8: $(TTAGGG)_4$ titration at 200 mM total ionic strength. Sequences were prepared in 10 mM phosphate buffer, pH 7.0 and the indicated amount of either K^+ and/or Na^+. Spectra were collected at 25 °C and strand concentrations were in the range of 3.28×10^{-5} to 5.09×10^{-5}.

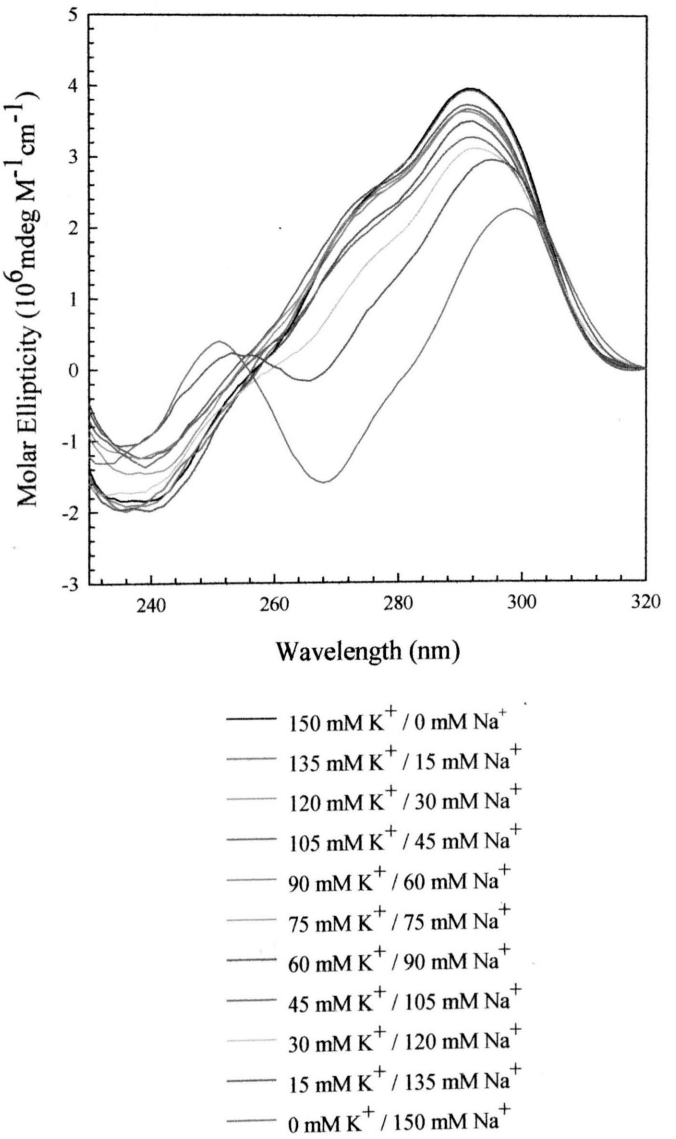

Figure 3.9: $(TTAGGG)_4$ titration at 150 mM total ionic strength. Sequences were prepared in 10 mM phosphate buffer, pH 7.0 and the indicated amount of either K^+ and/or Na^+. Spectra were collected at 25 °C and strand concentrations were in the range of 2.01×10^{-5} to 3.19×10^{-5}.

The results of this work tell us several things. 1) The lesser amount of K^+ ions required than Na^+ ions to form the potassium form of the quadruplex confirms that K^+ ions stabilize the structure to a greater extent than Na^+ ion, and 2) The duplication of results at different total ionic strengths tells us that the mole fraction of potassium in solution influences the folding topology of the quadruplex.

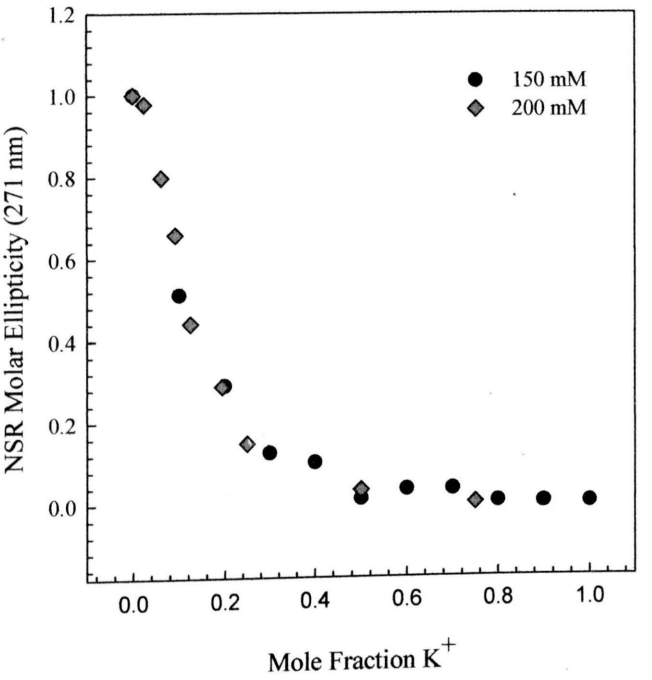

Figure 3.10: Transition plots. Analysis of Figure 3.9 gives the above plots for the transition from the Na^+ quadruplex to the K^+ quadruplex for $(TTAGGG)_4$. An explanation of NSR can be found in the legend for Figure 3.2.

CHAPTER IV

SUMMARY AND CONCLUSION

A thorough CD spectroscopic investigation of DNA oligomers of general sequence $(XXXGGG)_y$ (where X = A or T and y = 1, 2, 4, or 8) reveals that the conformations of the quadruplexes formed from these sequence are highly dependent upon sequence context, number of repeats and the identity of the cation present. Understanding the structures and stabilities that these types of sequences can fold into is a very complex task due to their high polymorphism.

The loop sequence context of quadruplexes has an apparent effect upon the conformation that the sequence will fold into because of the diverse loop orientations (strand reversal, lateral, or diagonal) possible. The loop sequence has no apparent effect upon the molecularity of the resulting quadruplex because all sequences with modified loops folded into an intramolecular, combination parallel and anti parallel quadruplex.

The number of times TTAGGG is repeated gives rise to quadruplexes of different molecularities (1, 2, or 4) and therefore different strand orientations (parallel, antiparallel or a combination of parallel and antiparallel). A similar effect is seen when changing the nature of the cation, different molecularities (1, 2, or 4) and therefore different strand orientations (parallel, antiparallel or a combination of parallel and antiparallel) are also observed when (TTAGGG)4 is in the presence of either K^+ or Na^+.

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