UNDERSTANDING THE CONFORMATIONS AND STABILITIES OF G-QUADRUPLEXES FORMED BY HUMAN TELOMERE SEQUENCE IN IDEAL AND LESS IDEAL SOLUTIONS

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DEDICATION

I dedicate my thesis work to my family and many friends. A special feeling of gratitude to my loving mother, Pushpa Devi, whose words of encouragement and push for tenacity ring in my ears.

I also dedicate this thesis to my many friends who have supported me throughout the process. I will always appreciate all they have done for helping me develop my communication and writing skills.

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ABSTRACT

VISHAL RAJAT SHARMA

UNDERSTANDING THE CONFORMATIONS AND STABILITIES
OF G-QUADRUPLEXES FORMED BY HUMAN TELOMERE
SEQUENCE IN IDEAL AND LESS IDEAL SOLUTION

AUGUST 2014

The G-rich termini of human chromosomes, called telomeres, have the ability to form unique DNA structures called G-Quadruplexes, which have been implicated in certain cancer types and, as a result, have drawn researchers' attention to study their structure inside the cell and thus consider them as potential targets for new anticancer therapies. Biophysical studies of these entities have been carried out under near ideal conditions, i.e., low phosphate concentration (e.g., 10mM), pH 7, low ionic strength (e.g., 115mM), and for optical studies, low concentration of DNA (e.g., 1×10^{-6} M). We, here, created less ideal conditions by using different osmolytes such as polyethylene glycol, acetonitrile, trifluoroethanol, and betaine. Our research focuses on the effect of these co-solutes on the structural and thermodynamic properties of telomeric DNA oligomers and report the characterization of $(TTAGGG)_x$, where x = 1, 2, or 4. In general, increasing the percentage of any osmolytes in solutions drives the equilibrium from unimolecular hybrid to multimolecular parallel conformation. In addition, Stability of parallel conformation also increases.

TABLE OF CONTENTS

Page

LIST OF EQUATIONS

Equations Page

LIST OF TABLES

Tables Page

LIST OF SCHEMES

Schemes Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Genomic stability and history of telomeres

Genomic stability is one of the major evolutionary processes which may have helped all the living species to survive. As it is known, the genomic information is contained in DNA in the form of chromosomes inside the delicate nucleus. As a result, there must be a shield or cap that protects the chromosomes form environmental and mechanical stresses. In early 1930's, Herman Miller and Barbara McClinktock observed that the ends of chromosomes behave differently than that of the rest of the chromosome and do not interact with each other. They called them telomeres[1] and were found to act as protective cap to the rest of chromosome. However, no extensive research was done on these entities right after their discovery, at least not until the discovery of double stranded duplex.

Different forms of DNA

The structure of deoxyribonucleic acid (DNA) as a double standard duplex was discovered by James D. Watson and Francis Crick in 1953[2]. According to their work, two strands run in opposite direction and are attached with each other via weak interactions, so called hydrogen bonds. Adenine makes two hydrogen bonds with thymine and guanine makes three hydrogen bonds with cytosine[2]. These H-bonds keep two strands

intact and makes a duplex structure as displayed in figure 1.1 A. Different forms of double stranded duplex have had also been observed since then and A-DNA, B-DNA, and Z-DNA are few examples of those.

All duplex forms of DNA originate at centromere and extend all the way to the end of the chromosomes until the 3' strand prolongs by itself and gives rise to structures other than double stranded duplex structure. In addition, several other DNA structures with unusual conformations have been discovered to be formed by the end of chromosomes.

As displayed in figure 1.1 D, a DNA hairpin is formed by single self-complementary DNA strand when it folds over and pair with the same strand. DNA hairpin follows Watson and Crick base pairing rules and contains, in addition, unpaired loop regions along with paired stem region[3].

Also, unlike two strands in duplex, three single strands of DNA can combine with each other to make a triplex[4]. The third strand aligns itself along the major grooves with the help of Hoogsteen bonding as depicted in figure 1.1 C. Furthermore, it was later found that extended 3' end of the chromosomes contains repeated bases of guanine and this isolated guanine rich strand achieve a unique conformation, so called G-quadruplex, different from the standard DNA duplex[5] which can be seen in figure 1.1 B.

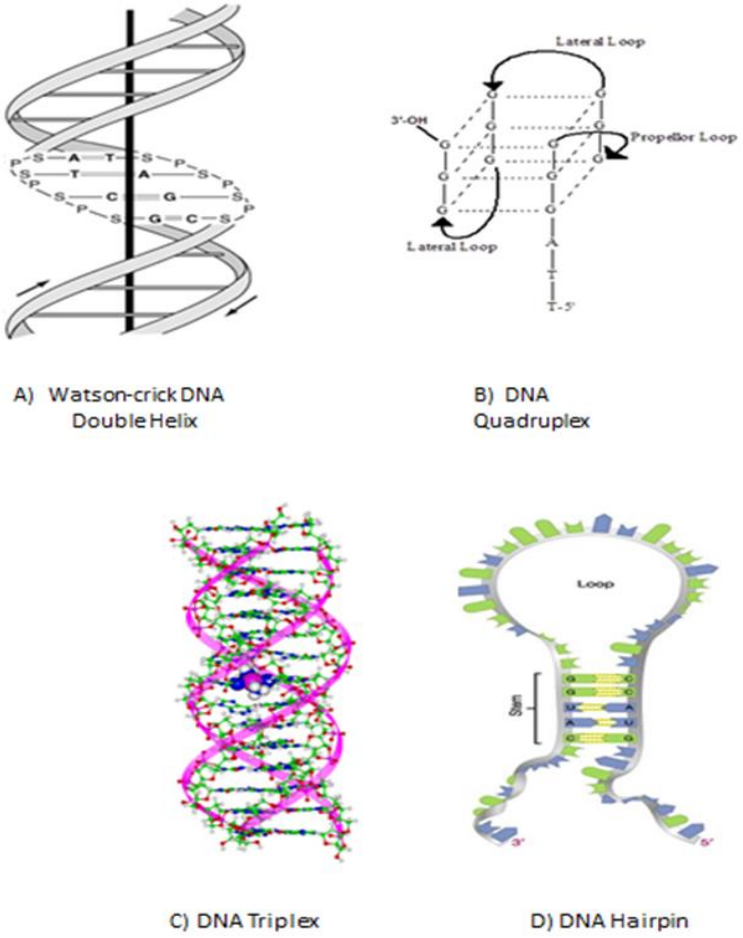


Figure 1.1: Different forms of DNAs' Secondary Structures.

Conformations of G-quadruplexes

Major work to understand the regions of telomeres, nature of telomerase, and their relations with cancer and age was done by Elizabeth H. Blackburn, Carol W. Greider, and Jack W. Szostak[1].

It was found that interactions inside the structure of G-quadruplex are also different than that of standard duplex DNA as each guanine (G) base in G-quadruplex pairs with two other guanine bases and make a squared tetrad structure[6] as displayed in figure 1.2. In addition, the presence of metal ion such as Na^+, K^+, or Mg^{2+} etc. in the center of tetrad provides extra stability to the quadruplex.

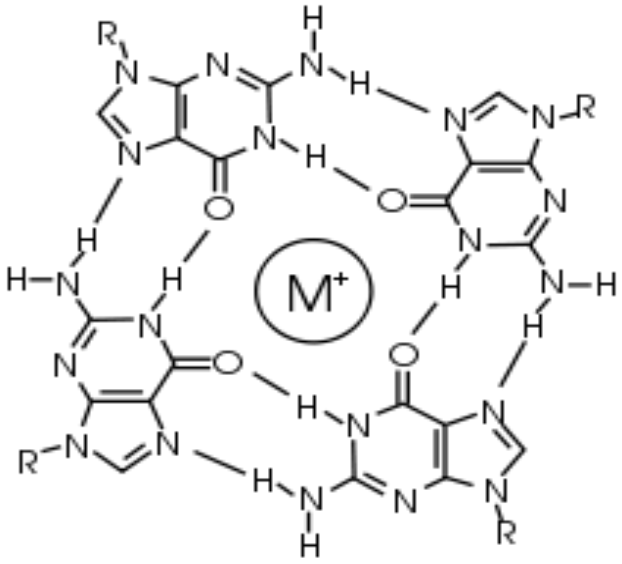


Figure 1.2: G-tetrad structure. Each Guanine pairs with two other Guanine bases to make a tetrad.

Quadruplexes can be formed from single stranded RNA or DNA as long as they contain at least four repeated sequence of two to four guanine bases. This unique structure not only protects chromosomes from degradation during mechanical and environmental stress; but it also aids in cell division and help cells to maintain their integrity. In 1973,

A. M. Olovnikov proposed a marginal theory and it states that chromosomal ends become shorter and shorter after each successive cell division of somatic cells, which means some bases get depleted each time a cell divides[11]. In order to keep the protective functions alive, the lost base pairs must be added. This particular task is accomplished by an enzyme called telomerase and it adds some of the lost bases every time a cell divides. According to Leonard Layflick's idea a somatic cell can divide only so many times until it eventually reaches a non-dividing state. These cells lose most of the bases of telomeric regions in order to reach their Layflick's limit[12]. However, cancer cells never reach that limit and continue on dividing because of uncontrolled telomerase activity keeps on adding bases to chromosomal telomeric regions. This is one of the reasons that studies on conformations of various G-rich strands have been popular for last four decades.

Circular Dichroism (CD) has been utilized to observe various conformations of quadruplexes. One long G-rich oligomer can fold itself back and forth with the help of lateral and propeller loops until the structure has four repeating segments, where guanines of one segment pairs with two other guanine bases of repeated segments of same strand[6-10] as displayed in figure 1.3 A. This intramolecular structure could be parallel, antiparallel or hybrid (mixer of both). In parallel conformation, all the repeated segments are connected with the help of propeller loops only and thus, points in one direction as showed in figure 1.3 B.

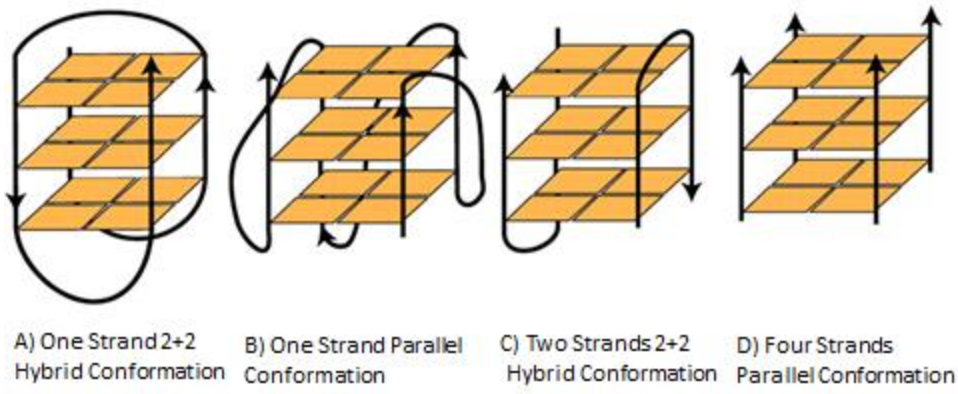


Figure 1.3: Different Conformations and Molecularities of G-Quadruplexes. Molecularity of one (A and B), two (C), and four (D). Arrow heads specify the orientations of segments or strand(s).

In addition, two strands as displayed in figure 1.3 C, which contain repeated sequence of guanine bases, can form a bimolecular hybrid type structure. A termolecular can also be formed if all the pairing segments come from four different strands[8] as depicted in figure 1.3 D. The presence of only the lateral or propeller or both loops potentially make different conformations of G-quadruplexes and as a result they would have different thermodynamic properties and thus, stabilities. So, it is paramount to know the exact conformation of the quadruplex that is present inside the cell before a drug can be designed to target these entities. To assimilate the cellular conditions, scientists around the globe have had been trying to use different osmolytes in stranded phosphate buffer of pH 7 and low K^+ concentration (115 mM). One of the earliest studies of DNA quadruplex was reported by Miyoshi et al in 2002 in the presence of a crowding agent PEG 200 (polyethylene glycol). They reported that increasing concentrations of PEG 200 induces a conformational transition in the sequence $G_4T_4G_4$ from a bimolecular anti

parallel quadruplex to a termolecular parallel quadruplex and the transition is completed by 40% PEG 200[13]. In addition, in 2007, Xue et al reported the complete conversion of human telomere sequence $G_3(T_2AG_3)_3$ from a unimolecular parallel/antiparallel quadruplex to parallel stranded structure at 40% PEG[14]. Heddi and Phan investigated the effect of several crowding agents on the conformation of DNA sequence containing a $G_3(TTAG_3)_3$ core using both CD and NMR approaches. They found that the percentage of PEG 200 required to induce a high order structure depended upon the particular sequence context of the base flanking the $G_3(TTAG_3)_3$ core[15]. While other studies have used different quadruplex forming DNA sequences, most have used 40% as the conformational transition inducer[16-20].

Questions and possible answers

Is PEG really a good crowding agent and does it assimilate complete cell conditions? If so, what molecular weights and percentage of PEG will integrate that environment? How do the changes in molecular weight of PEG affect the conformation and stability? Although PEG 200 is commonly used, it may or may not be a good crowding agent because of its low molecular weight. It is clearly an osmolyte that will impact the activity of water. As a result, the effect of higher molecular weight PEG (2000 to 10,000) on the conformations and stabilities of DNA sequence with one, two, or four repeats of TTAGGG needs to be investigated. In addition, some other osmolytes with known water disruptive properties such as trifluoroethanol, acetonitrile and betaine will also be used to cross check those results with polyethylene glycol.

Spectroscopic Theories

To accomplish these aims, Circular Dichroism will be engaged to study the changes in conformations and stability (T_m values), Differential Scanning Calorimetry to determine the stability (T_m values), UV-Vis Spectroscopy to figure out the concentrations of oligomers, and Osmometer to calculate the milliosmolality of different concentration of osmolytes.

Circular Dichroism is used to measure the difference in absorption of left and right circularly polarized light. The difference between the absorption of different amount of the left and right handed light is called ellipticity and can be calculated as:

$$\Theta = \Theta_\ell - \Theta_r \qquad 1.1$$

Where Θ is ellipticity, Θ_ℓ is left handed absorption, and Θ_r is right handed absorption of plane polarized light. The molecule must be a chiral in order to employ this technique. The instrument can be used to determine the different types of DNA secondary structures as different secondary structures absorbs polarized light of different wavelength. The data obtained from CD are often presented as a plot of molar ellipticity versus wavelength. The concentration of the DNA is taken into account when calculating molar ellipticity. The relationship between molar ellipticity, ellipticity, concentration of DNA, and path length of cuvette is given by:

$$[\Theta] = \frac{\Theta}{c\ell} \qquad 1.2$$

Where [Θ] is molar ellipticity, Θ is ellipticity, c is the concentration of DNA sample, and ℓ is the path length of the cuvette.

DNA concentration is calculated using Uv-Vis spectroscopy. DNA absorbs light between a range of 240-280 nm with a maximum absorption around 260 nm. The molar extinction coefficient (Ɛ) and wavelength of maximum absorbance (λ_{max}) are dependent upon several different factors including base composition, base-pairing interaction, the salt concentration of the solution, and the pH of solution. We use Beer's Law to determine the concentration of DNA in our samples as:

$$A = \varepsilon \ell c \qquad 1.3$$

Where A is the absorbance, Ɛ is molar extinction coefficient, ℓ is the path length of the cuvette, and c is the molar concentration. If the molar extinction coefficient of the sample and the path length of the cuvette are known, the concentration of sample can be calculated after obtaining value of absorbance by Uv-Vis spectrophotometer.

Differential Scanning Calorimetry can be used to determine the T_m value of DNA sample and other parameters of Gibbs free energy equation. A concentrated sample of DNA is loaded and multiple heating and cooling scans are obtained. The overlapping of

all the heating scans gives an area under the curve and system use it to calculate the T_m value.

To investigate the activity of water on the conformation of $(TTAGGG)_4$, we are using Osmometer to determine the milliosmolality per kilogram of different osmolytes at their various concentrations. A plot of milliosmolality versus percent osmolyte is used to compare the osmotic pressure of all different osmolytes.

CHAPTER II

MATERIALS AND METHODS

Preparation of buffer

10X potassium buffers (150 mM K^+, 100 mM PO_4^{-3}, and 1.0 mM EDTA) at pH 7 were prepared by dissolving 6.8054 g KH_2PO_4 (VWR International Lot # 46032627), 8.71 g K_2HPO_4 (VWR International Lot # 46205641), 0.372 g EDTA (EMD Chemicals Lot# 45166714), and 37.2757 g KCl (VWR International Lot # 46100627) in 1 L deionized water. 1X potassium buffer (115 mM K^+) was prepared by adding 100 ml 10X K^+ buffer and 100 ml 1M KCl in 800 ml deionized water. All buffers were filtered through a 0.45 μm Millipore filter and degassed before being stored for use.

Preparation of crowding agents

The polyethylene glycol, a crowding agent, of molecular weights 2000, 6000, and 10000 were used to make various solutions of different percentage by weight/volume by dissolving appropriate amount in 115 mM K^+ phosphate buffer. 60% PEG 6000 was prepared by dissolving 60.0038 g of solid PEG 6000 (Alfa Aesar Lot # 10167753) in 100ml buffer. The solution was stirred for about 5 hours before all the contents of PEG 6000 were completely dissolved. Similarly, 40% PEG 2000 was prepared by dissolving 40.0098 g of solid PEG 2000 (Alfa Aesar Lot # 10172606) in 100 ml buffer. In addition, 40% PEG 10000 was made by dissolving 20.0035 g of solid PEG 10000 (Alfa Aesar Lot

10166724) in 50 ml phosphate buffer. All the above solutions were diluted to lower concentration by using buffer solution.

All HPLC- purified oligomers, $(TTAGGG)_x$, where x = 1, 2, or 4, were purchased from biosynthesis Inc. (Lewisville, TX) and used without further purification. All sequences were reconstituted in about 1 ml of deionized water and heated to 95 ^oC before they were left to cool down to room temperature for 24 hours. The samples were then fragmented into 5 eppendorf tubes. The water was evaporated off using a Savant DNA 110 speed vacuum and approximate 600 µl of buffer with no PEG or buffer with different concentration of PEG of appropriate molecular weight was added. The CD melts of freshly made samples were obtained. The CD scans of the same samples after waiting for more 24 hours were also obtained.

Osmometer

An Advanced Instruments Inc. Osmometer model 3320 was used to measure the milliosmolality of the solution containing different concentrations of osmolytes.

UV-Vis Spectroscopy

A Varian Cary 100 Bio model (Varian Associates, Palo Alto, CA) UV/Vis spectrometer was used to measure the concentration of the samples used in Circular Dichroism. Appropriate dilutions of each sample were made and run, in 5 mm square quartz cuvettes, from 320 – 220 nm at 25 and 95 ^oC with the appropriate baseline

subtracted. Beer's Lambert law was used to calculate the concentrations of samples at 25 ^{o}C. Molar extinction coefficient (see table below) and absorption at 260 nm was used.

Table 2.1: Values of molar extinction coefficients for different oligomers.

Number of Repeated Effects	
Oligomers	Є (mol^{-1} bases cm^{-1})
$(TTAGGG)_4$	244600
$(TTAGGG)_2$	122400
$(TTAGGG)_1$	61300
$(CCCTAA)_4$	220400

Circular Dichroism

All circular dichroism studies were carried out using an Olis RSM 1000 spectrophotometer (Olis, Inc. Athens, GA). Data was collected over the range of 320nm to 220 nm at temperatures range from 25 – 95 ^{o}C with 5 ^{o}C increment after each scan with an integration time of 3 seconds. All the samples including baseline were run in 1 mm circular quartz cuvette. The baseline of different PEG concentration was run individually and subtracted from each spectrum. Olis Global works and sigma plot version 11 was used to process and analyze the data.

CHAPTER III

RESULTS AND DISCUSSIONS

Selection of oligomer sequence

Our aims were to study the effect of the lengths of human telomeric sequences on the conformation and stability of their resultant quadruplexes. In addition, we focused on the shift of the equilibrium between the hybrid and parallel conformations of quadruplexes in several concentrations of different osmolytes. We chose to study three different G-rich sequences with various lengths: TTAGGG, $(TTAGGG)_2$, and $(TTAGGG)_4$ referred as HTEL1 M, HTEL1 D, and HTEL1 respectively and one C-rich sequence $(CCCTAA)_4$ referred as HTEL1 C as shown in Table 1. In addition, we also studied the effect of the presence of osmolytes on the stability of the double stranded DNA formed after mixing 1:1 molar ratio of HTEL1 and HTEL1 C.

Quadruplexes formed by these different sequences in phosphate buffer of pH~7 containing K^+ ions in the absence or presence of osmolytes or co-solutes will be compared. The initial idea to use osmolytes was to create cellular-like conditions. However, do we really know how does the inside of a cell looks-like? Answer to this question may not be as easy as it sounds. As a result, we decided to use multiple osmolytes and compare their effect on the transformation of quadruplexes conformations

and their relative stabilities. Different conformations of quadruplexes have unique CD spectra and can be easily differentiated among themselves. Antiparallel quadruplexes have a characteristics CD spectra and can be identified with a positive peak at about 300 nm, a negative peak at about 270 nm, a positive peak at about 250 nm, and another negative peak at about 240 nm. In addition, parallel quadruplexes have a unique CD spectrum that shows a positive peak at about 260 nm and a negative peak at about 240 nm. Furthermore, hybrid quadruplex can be identified with a positive peak at 292 nm, a shoulder at about 275 nm, and a negative peak at about 250 nm.

Table 3.1: Different Oligomers with Various Lengths

Name	Sequence (5’ to 3’)
HTEL1 M	TTAGGG
HTEL1 D	TTAGGGTTAGGG
HTEL1	TTAGGGTTAGGGTTAGGGTTAGGG
HTEL1 C	CCCTAACCCTAACCCTAACCCTAA
Duplex	(TTAGGGTTAGGGTTAGGGTTAGGG) (CCCTAACCCTAACCCTAACCCTAA)

The reported DNA oligomers could potentially form quadruplexes of different conformations. As a result, we decided to start our work by comparing the different quadruplexes formed by these three DNA sequences of different length in similar conditions of solution containing standard phosphate buffer at pH 7.0 and 115 mM K^+.

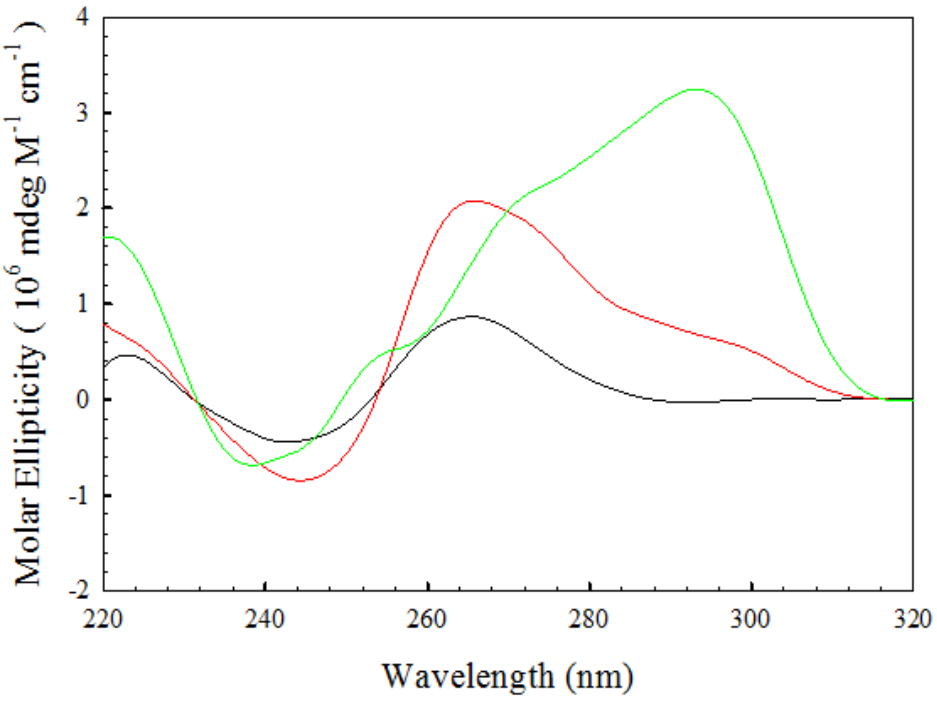


Figure 3.1: Overlays of CD spectra of $(TTAGGG)_x$ oligomers. Spectra of $(TTAGGG)_4$ (green), $(TTAGGG)_2$ (red), and (TTAGGG) (black) were taken in standard phosphate buffer at pH 7.0 and 115 mM K^+ at 25 ^oC.

The CD spectrum of $(TTAGGG)_4$ (green), also called HTEL1, has a major positive peak, a shoulder, and a negative peak at approximate 292 nm, 270 nm, and 240 nm

respectively. This particular CD scan is representative of a quadruplex with a hybrid conformation. In addition, positive and negative peaks were observed at about 260 nm and 245 nm respectively for the CD spectrum of $(TTAGGG)_2$ (red), also referred HTEL1 D. Similarly, the CD spectrum of (TTAGGG) (black), also called HTEL1 M, has a positive and a negative peak at about 260nm and 245 nm respectively. The CD spectra of both (TTAGGG) and $(TTAGGG)_2$ have characteristic peaks at 260 nm indicating that the quadruplexes formed by both of these sequences had adopted parallel conformations. However, the relative intensity of the peak for (TTAGGG) was significantly lower than that of $(TTAGGG)_2$ sequence. There could be a possibility of self-assembly in $(TTAGGG)_2$. However, it is beyond the reach of CD. NMR and gel electrophoresis studies on these sequences under same solution conditions might be a possible solution for this problem. However, we were able to confirm the formation of same conformation of quadruplexes for two sequences under same conditions. In order to determine the stabilities of formed quadruplexes of respective sequences, we collected the CD spectra in standard phosphate buffer of pH 7.0 and 115 mM K^+ as a function of temperature. The scans were taken after every 5 ^oC with an incubation period of 5 minutes from 25 ^oC to 95 ^oC. The melting CD spectra of all three sequences (HTEL1, HTEL1 D and HTEL1 M) and the duplex made by mixing 1:1 molar of $(TTAGGG)_4$ and $(CCCTAA)_4$ are displayed in Figure 3.2. As displayed, intensity of the dominant peaks at 292 nm, 265 nm, 265 nm and 270 nm of HTEL1, HTEL1 D, HTEL1 M, and duplex respectively gradually decrease as the temperature is increased. The last few spectra at temperatures 85 ^oC, 90

^{o}C, and 95 ^{o}C of respective HTEL1, HTEL1 M, and HTEL1 D were nearly superimposed indicating that the oligomers were fully unfolded by 85 ^{o}C. However, CD spectra of duplex at these temperatures were not superimposed suggesting that double stranded DNA structure still existed even at the highest temperature possible.

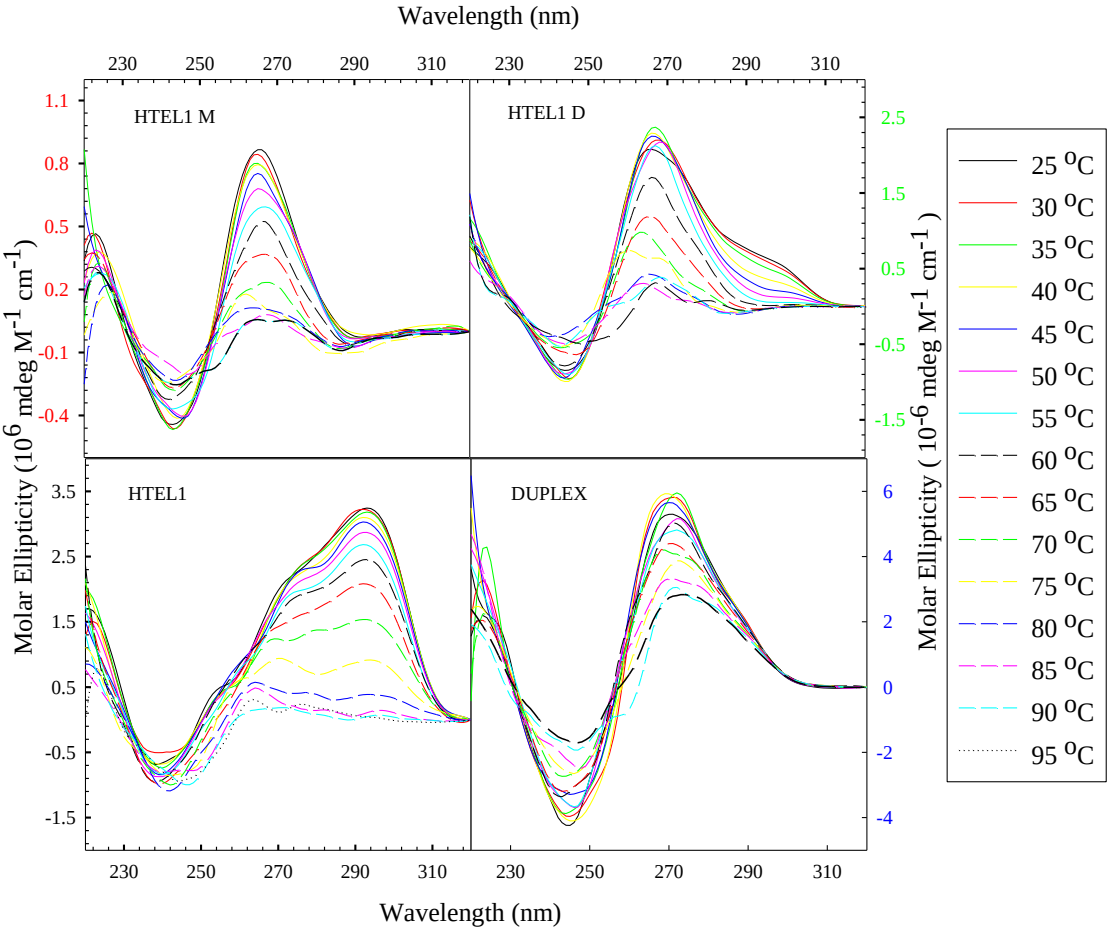


Figure 3.2: CD optical melting spectra of $(TTAGGG)_x$ oligomers and duplex. Spectra were taken in standard potassium phosphate buffer (115 mM K^+) (Spectra drawn on different scales to better observe changes).

This inferred that double stranded duplex was more stable than any of the quadruplexes formed by any of the sequences under the same conditions of solutions and thus confirmed that interactions other than just H-bonds also play an important role in the stability.

However, this particular potassium phosphate buffer solution may not be adequate to simulate cellular like conditions. Despite the presence of same phosphate buffer inside human cells, existence of various proteins and organelles could possibly divert the nature of solution to a solution that behaves more like a gel. We attempted to create similar conditions by adding different osmolytes or cosolutes one by one in original phosphate buffer.

Effect of PEG on conformations and stabilities

We shall be studying the equilibrium shift of conformations of quadruplexes and their stabilities in different concentrations of osmolytes. The very first osmolyte used was polyethylene glycol (PEG) of different molecular weights. We increased the concentration of PEG 6000 in small increments in order to determine its effect on the conformational shift of quadruplexes and their stabilities. The CD optical melting spectra of HTEL1 M were compared in three different solution conditions 1) phosphate buffer only; 2) 10% PEG; and 3) 20% PEG. Different concentrations of PEG were prepared in original buffer solution by weight/volume ratio. Melting CD spectra of HTEL1 M in three different conditions can be seen in figure 3.3. All three graphs were drawn on

different scales in order to see the effect of increasing temperature clearly. Both in the absence and presence of PEG, HTEL1 M has a positive peak at 270 nm which confirms the potential existence of quadruplexes of only parallel conformation. As the temperature is increased, HTEL1 M undergoes a transition from the fully folded state at 25 ^{o}C to the fully unfolded state at 95 ^{o}C.

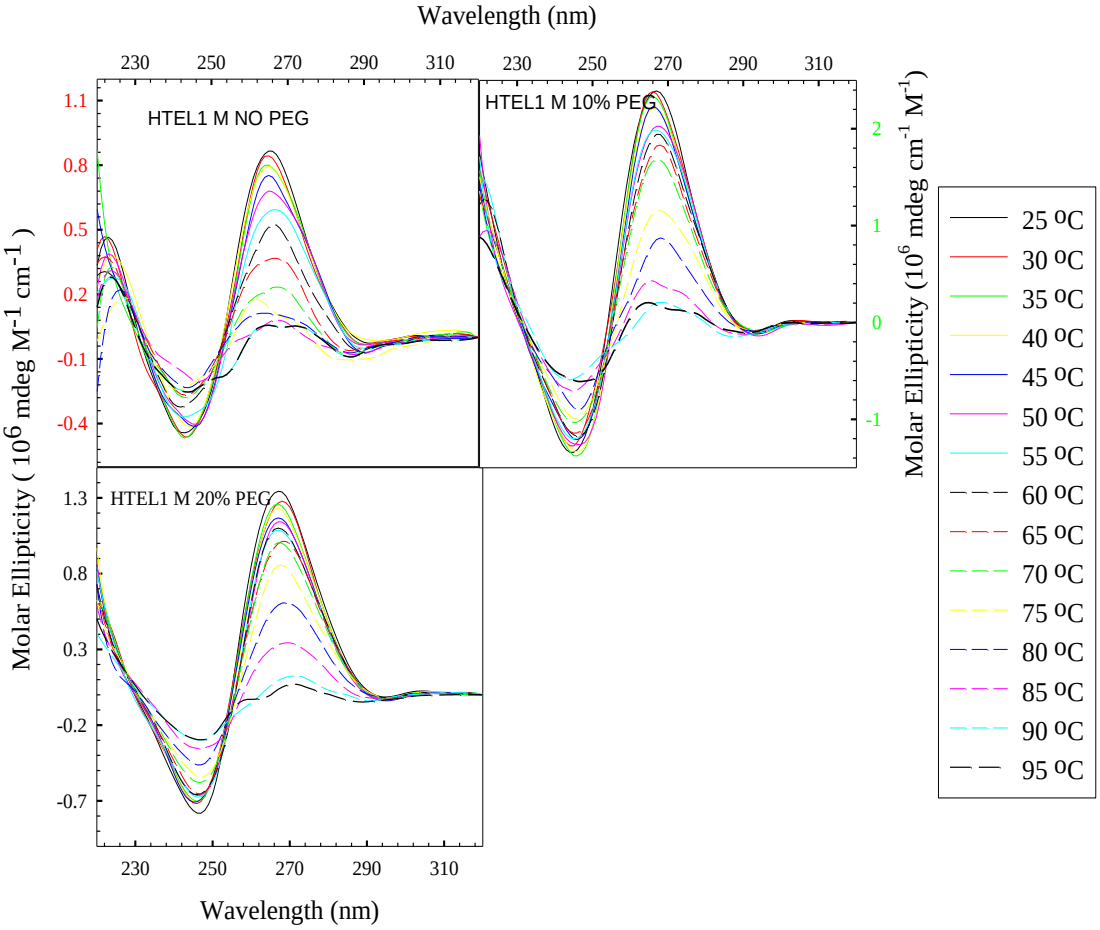


Figure 3.3: Typical CD optical melting spectra of TTAGGG. Spectra were taken in standard potassium phosphate buffer (115 mM K^{+}) as a function of percent weight PEG 6,000. (Graphs drawn on different scales to better observe gradual changes)

The spectra recorded at 90 ^{o}C and 95 ^{o}C nearly overlay suggesting the presence of the single stranded oligomers. Thus, there is not a significant difference in the stabilities of the quadruplexes formed in higher concentrations of PEG.

Melting studies of HTEL1 D $(TTAGGG)_2$ were also executed in various concentrations of PEG 6000. The CD spectra of HTEL1 D contain minor shoulders at 290 nm and dominant peaks at 265 nm in standard phosphate buffer which represents the signature scan of quadruplexes of parallel conformations. The characteristic spectrum remains the same as the concentration of PEG is increased. This suggests that the addition of the PEG did not trigger any conformational changes in original formed quadruplexes.

Figure 3.4 displays CD melting spectra of HTEL1 D as a function of temperature in four PEG 6,000 concentrations 1) no PEG, 2) 2.5% PEG, 3) 5% PEG, and 4) 7.5% PEG. A steady and gradual decrease in the intensity of the peak at about 270 nm is observed with the increase of temperature.

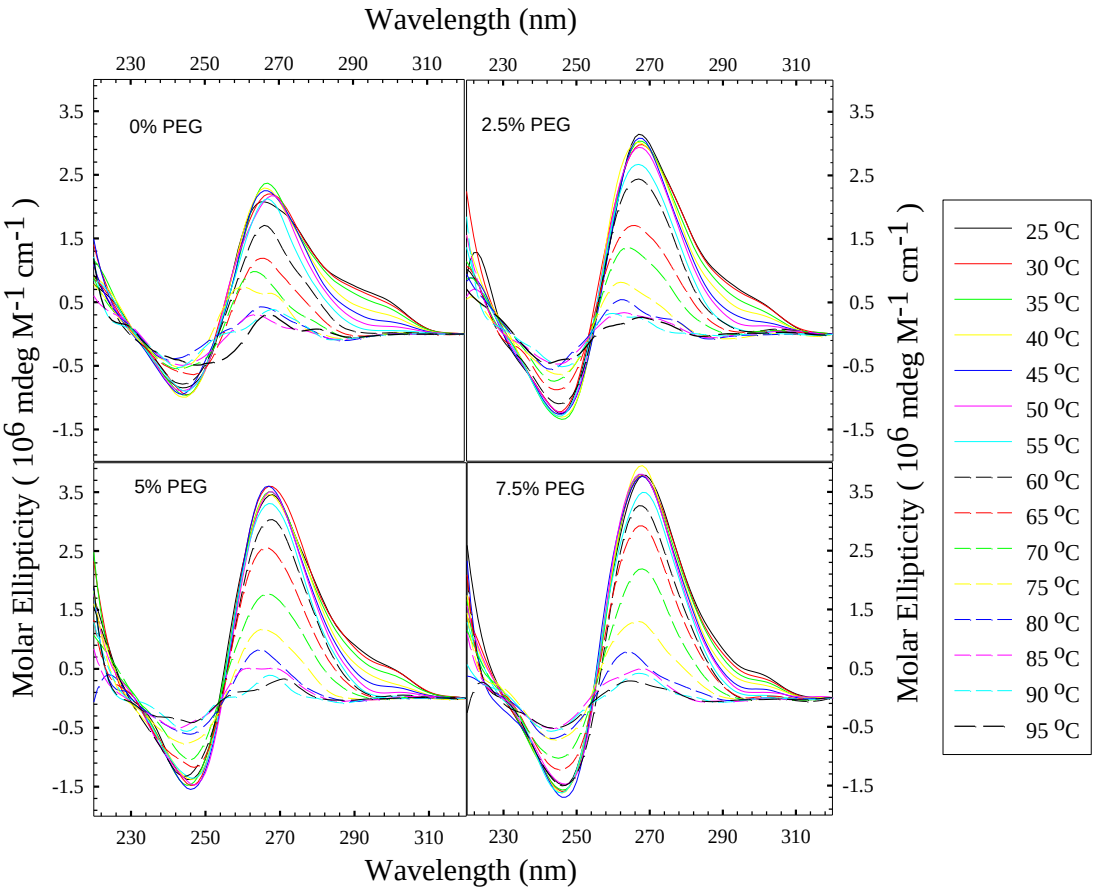


Figure 3.4: CD optical melting spectra of $(TTAGGG)_2$ from 0% to 7.5% PEG. Spectra were taken in standard potassium phosphate buffer (115 mM K^+) as a function of percent weight PEG 6,000.

The CD optical melting spectra of HTEL1 D as a function of temperature was also taken in 10% PEG, 12.5% PEG, 15% PEG, 17.5% PEG, 20% PEG, 25% PEG, 30% PEG, and 40% PEG as displayed in Figures 3.5 and 3.6.

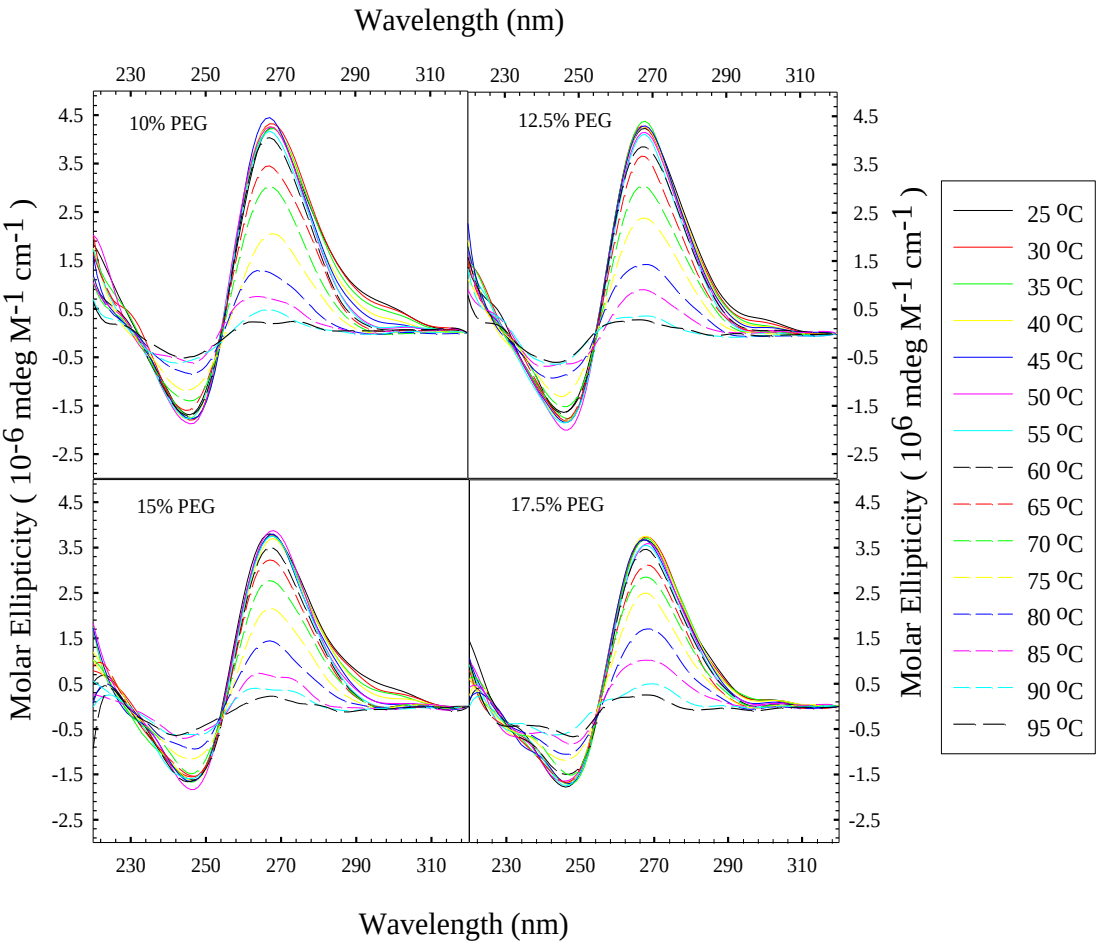


Figure 3.5: CD optical melting spectra of $(TTAGGG)_2$ from 10% to 17.5% PEG. Spectra were taken in standard potassium phosphate buffer (115 mM K^+) as a function of percent weight PEG 6,000.

The CD optical melting spectra indicate that HTEL1 D in solutions with concentration of PEG from 0 to 20% undergo transitions from a fully folded states at 25 ^oC to the fully unfolded states at 95 ^oC as previously seen in HTEL1 M. Similar to CD

spectra of HTEL1 M, spectra of HTEL1 D at 85 ^{o}C, 90 ^{o}C, and 95 ^{o}C are also superimposable indicating that the oligomers is fully unfolded to a single strand structure.

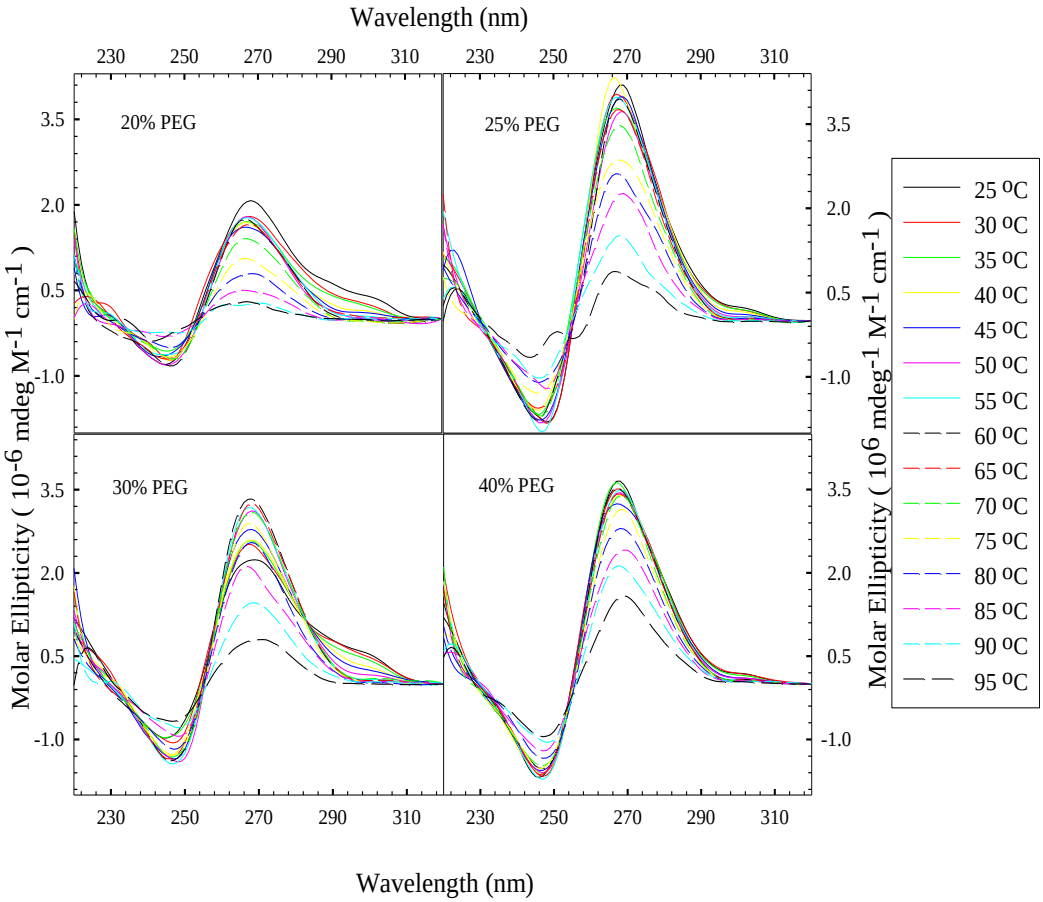


Figure 3.6: CD optical melting spectra of $(TTAGGG)_2$ from 20% to 40% PEG. Spectra were taken in standard potassium phosphate buffer (115 mM K^+) as a function of percent weight PEG 6,000.

However, the spectra of HTEL1 D at 90 ^{o}C and 95 ^{o}C in solutions greater than 20% PEG are not superimposable suggesting that oligomer might not be fully unfolded even at these temperatures. This suggests that as the concentration of PEG is increased, the

stability of quadruplexes of parallel conformation also increases relative to ones formed by HTEL1 M. CD spectra of HTEL1 M and HTEL1 D are very similar in regards to the characteristics and positions of the peaks of all their spectra in both presence and absence of polyethylene glycol. Only the relative intensities of the peaks are different for them.

The above studies suggest that both HTEL1 M and HTEL1 D form similar quadruplexes even in various conditions of solutions. As a result, we shifted our attention to HTEL1 (TTAGGGTTAGGGTTAGGTTAGGG) which is a longer sequence than both HTEL1 D and HTEL1 M and more comparable to the wild sequence present in human telomeric region. HTEL1's behavior is more dramatic than HTEL1 D and HTEL1 M in solutions of different conditions. In standard potassium phosphate buffer, the CD spectrum of HTEL1 (black scan) at 25 ^{o}C entails a positive peak, a shoulder, and a negative peak at 292 nm, 275 nm, and 240 nm respectively. This particular spectrum represents a quadruplex of hybrid conformation. As the concentration of the PEG 6,000 is slowly increased from 0% to 20%, the intensity of the peak and the shoulder at 292 nm and 275 nm start to decrease and increase respectively as shown in figure 3.7. The peak at 292 nm slowly diminishes while the shoulder at 272 nm develops into a very intense peak. This change implies a gradual transition from normal CD spectrum of a quadruplexes of hybrid conformation to one which was very similar to that observed for quadruplexes of parallel standard conformation.

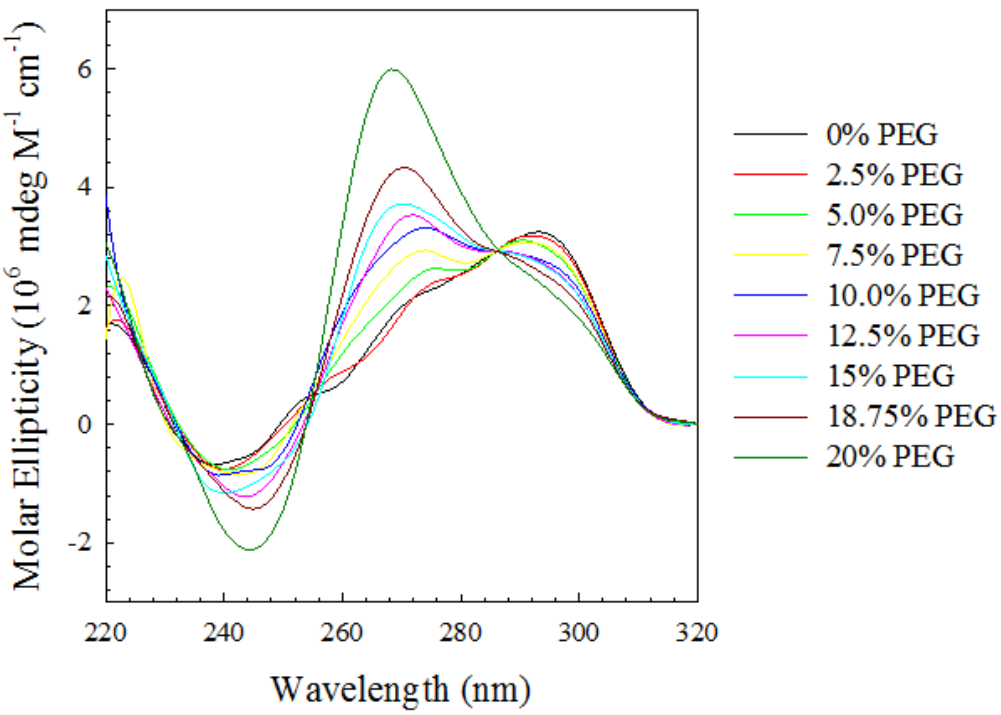


Figure 3.7: CD spectra of $(TTAGGG)_4$. Spectra were taken in standard phosphate buffer (pH 7.0, 115 mM K^+) at 25 ^oC in the absence and presence of increasing weight percent of PEG 6,000.

At about 10% PEG (blue spectrum), intensities of the peaks at 292 nm and 270 nm are almost identical suggesting that, at this particular concentration of PEG, there is an equal population of two quadruplexes. With further increases in the concentration of PEG, the intensity of peak at 272 nm was increases dramatically suggesting that the quadruplexes of parallel conformation have outnumbered the quadruplexes of hybrid conformation. At about 20% PEG (green spectrum), the dominant peak at 272 nm is indicative of complete transition. Furthermore, all the CD spectra pass through a well-defined isoelliptic point at

282 nm as shown in Figure 3.7. This suggests that the transformation of conformations of the quadruplex follows two state transitions. The effect of molecular weight of PEG on the conformational change was also studied. PEG of three different molecular weights 2000, 6000, and 10000 were chosen to carry out the experiments.

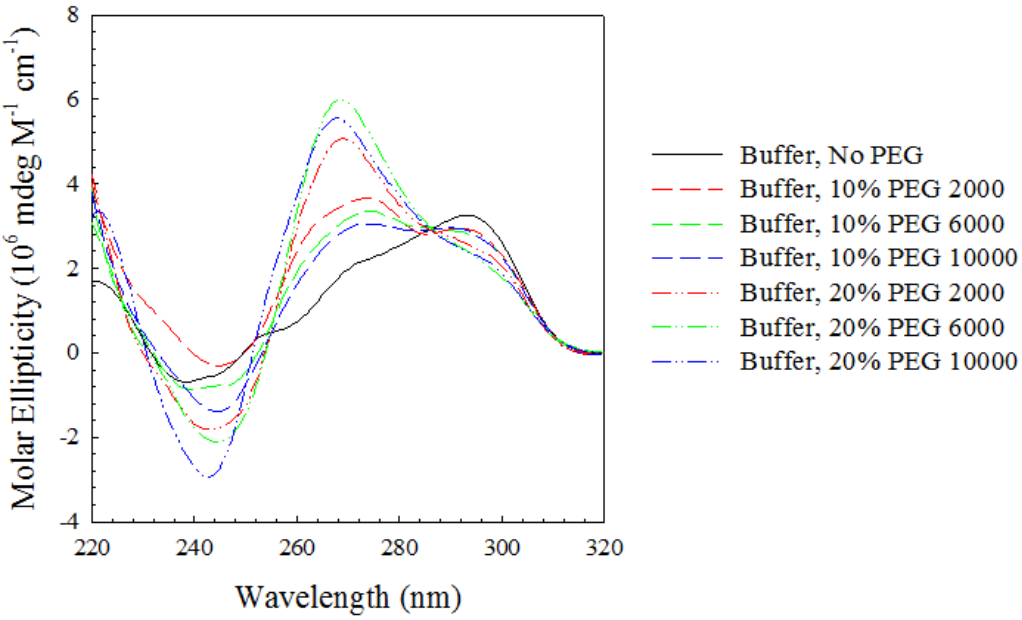


Figure 3.8: CD spectra of $(TTAGGG)_4$ in different PEG. Spectra were taken at 25 ^oC in standard potassium phosphate buffer of 115 mM K^+ in the absence or presence of PEG 2,000, PEG 6,000, and PEG 10,000.

Figure 3.8 displays the CD spectra of $(TTAGGG)_4$ at 25 ^oC in standard phosphate buffer with 115 mM K^+ in the presence or absence of PEG of different molecular weights and concentrations. The CD spectrum of HTEL1 in the absence of PEG (black line) has the peak and a shoulder at 292 nm and 272 nm respectively, representing a characteristic

scan of unimolecular quadruplex of hybrid conformation. The spectra in10% PEG 2000 (Red Dash), 10% PEG 6000 (Green Dash), and 10% PEG 10000 (Blue Dash) at 25 ^{0}C are very similar with minor differences in the intensities of their peaks at 272 nm. The peak of HTEL1 in 10% PEG 2000 is the most intense whereas the peak of 10% PEG 10000 is lowest in intensity. This suggests that there might be a greater transformation of the conformations in 10% PEG 2000 solution. Furthermore, the CD spectra of HTEL1 in 20% PEG 2000 (Red Dash Dotted), 20% PEG 6000 (Green Dash Dotted), and 20% PEG 10000 (Blue Dash Dotted) have dominant peaks at 270 nm with minor differences in their intensities and troughs at 242 nm. The presence of the dominant peak at 270 nm implies that there is a complete transformation of the conformation from a unimolecular hybrid quadruplex to a termolecular or multimolecular parallel quadruplex for all the molecular weights of PEG. In addition, all the spectra again pass through a well-defined isoelliptic point at about 286 nm. It is safe to say that the conformational change depends mainly on the concentration of PEG and is fairly independent of the molecular weight of PEG.

The next task was to determine the stabilities of quadruplexes in the presence and absence of PEG. In order to determine the stability, melting studies were done with slow increments of increasing PEG concentration. As a result, CD spectra of HTEL1 were taken in different PEG 6000 concentrations as a function of temperature and are displayed in figures 3.9, 3.10, and 3.11. The spectra of each sample of HTEL1 were taken every 5 ^{o}C with an incubation period of 5 minutes with a temperature range from 25 ^{o}C to 95 ^{o}C.

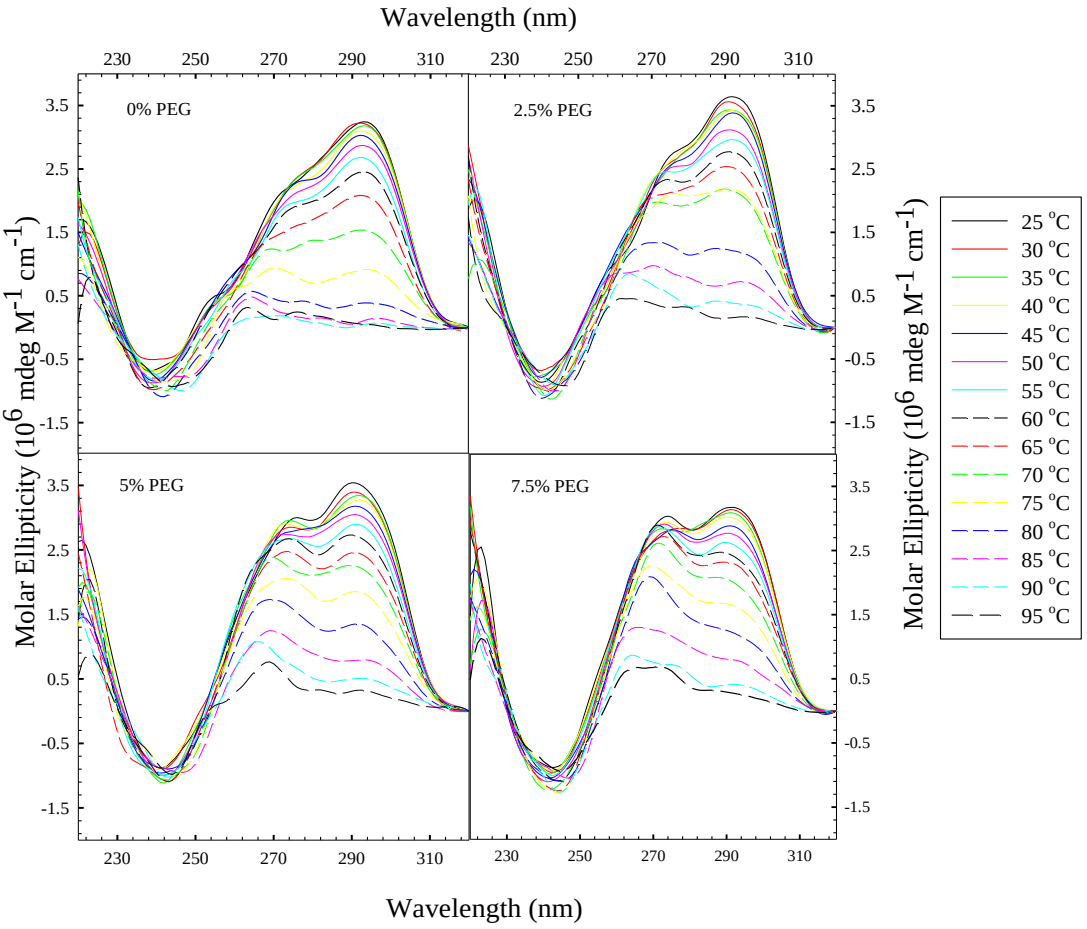


Figure 3.9: CD optical melting spectra of $(TTAGGG)_4$ from 0% to 7.5% PEG. Spectra were taken in standard potassium phosphate buffer (115 mM K^+) as a function of percent weight PEG 6000.

As the temperature of the sample increases, a slow and gradual decrease of both the peak at 292 nm and the shoulder 270 nm was observed. In addition, the spectra of HTEL1 in 0% PEG (Buffer) at 85 ^{0}C, 90 ^{0}C, and 95 ^{0}C overlay with each other implying that the quadruplex was completely melted to its single stranded state.

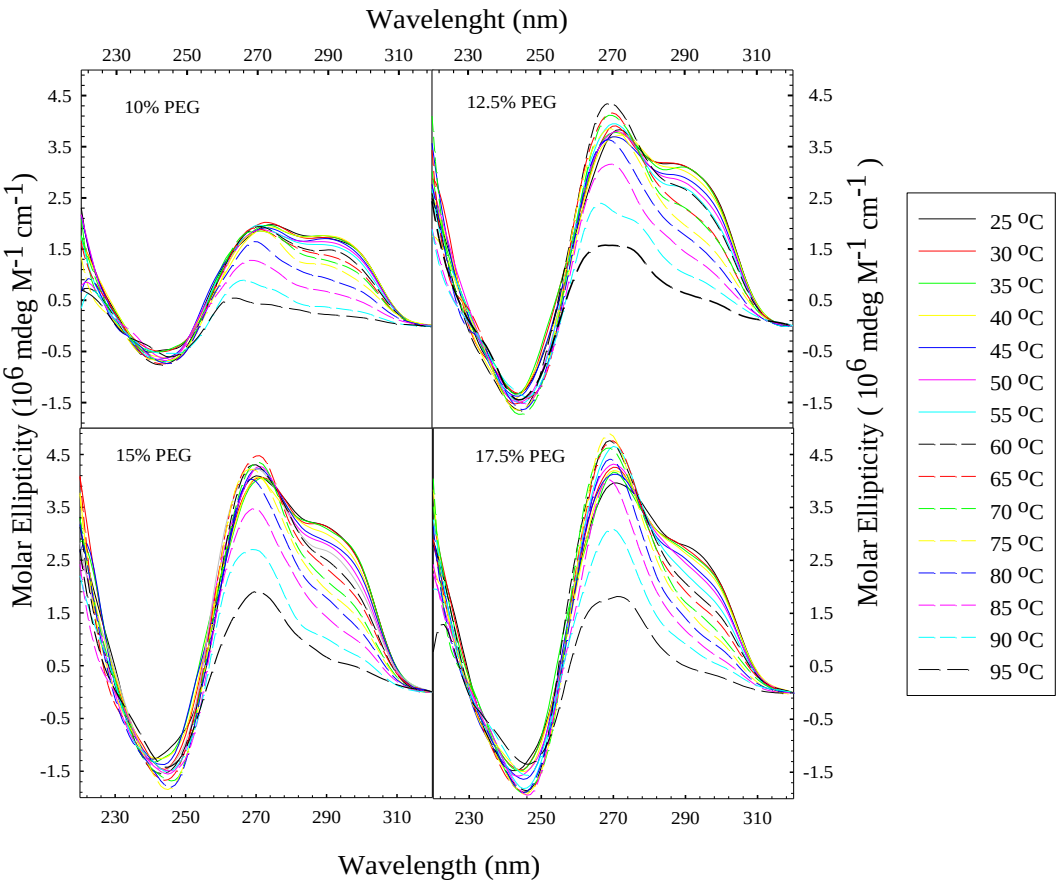


Figure 3.10: CD optical melting spectra of $(TTAGGG)_4$ from 10% to 17.5% PEG. Spectra were taken in standard potassium phosphate buffer (115 mM K^+) as a function of percent weight PEG 6,000.

The equilibrium shift from hybrid conformation to parallel conformation of the quadruplex was observed as the concentration of PEG was increased. The analogous and steady decrease in the intensities of the peaks was also observed in all the concentration of PEG. However, intensity of peak at 272 nm was increased with increase in PEG concentrations.CD melting spectra of HTEL1 in 5% and 7.5% PEG did not have any

overlapping of scans at 85 ^{0}C, 90 ^{0}C, and 95 ^{0}C evidencing the presence of some intact quadruplex even at the highest temperature. This implies that as the concentration of the PEG is increased, the stability of quadruplex was also increases.

The CD melting spectra of HTEL1 in 10% PEG displays a similar trend as that of HTEL1 in buffer as the temperature is increased. However, a peak at 270 nm with minor intensity was found even at 95 ^{o}C suggesting a possible increase in the stability. Furthermore, the intensity of this particular peak keeps increasing as the concentration of the PEG increases to 12.5%, 15%, 17.5%, 20%, 25%, 30%, and 40% indicative of an increase of stability of quadruplex. In addition, as displayed in the figures, when the PEG percentage was increased the intensity of the peak at 272 nm was dramatically increased, whereas, the intensity of the peak at 292 nm faded away. At about 20% PEG solution, a very dominant peak at 272 nm and a minor shoulder at 292 nm suggest a potential complete transformation of the conformation of quadruplex. In addition, a very intense peak was reported at 270 nm at the highest temperature 95 ^{o}C inferring the presence of significant amount of parallel conformation quadruplex. The intensity of the same peak at 95 ^{o}C increases in the spectra at 25% and 30% PEG. However, the intensity of the peak at 270 nm at 25 ^{0}C decreases when recorded in the solutions containing PEG concentration of more than 30%. The reason for this behavior could possibly be the presence of too much PEG in the solution and not having enough space for the quadruplex.

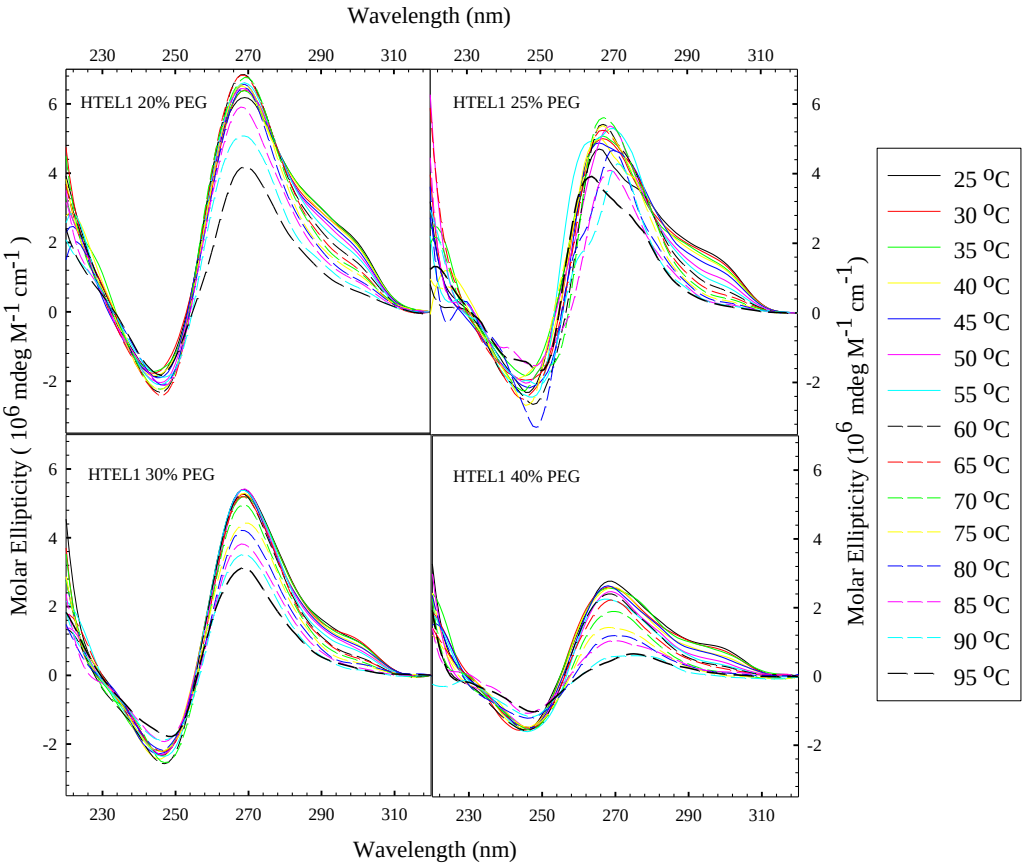


Figure 3.11: CD optical melting spectra of $(TTAGGG)_4$ from 20% to 40% PEG. Spectra were taken in standard potassium phosphate buffer (115 mM K^+) as a function of percent weight PEG 6,000.

It is safe to assume that the conformational change of the quadruplex is fairly independent of the molecular weight of PEG. Furthermore, as the concentration of the PEG is increased, the stability of the formed quadruplex increases along with the shift in equilibrium. Figure 3.12 displays the CD spectra of HTEL1 at 95 ^{o}C at various

concentrations of PEG 6000. As the concentration of PEG 6000 increases, the intensity of the peak at 270 nm at 95 ^{o}C also increases.

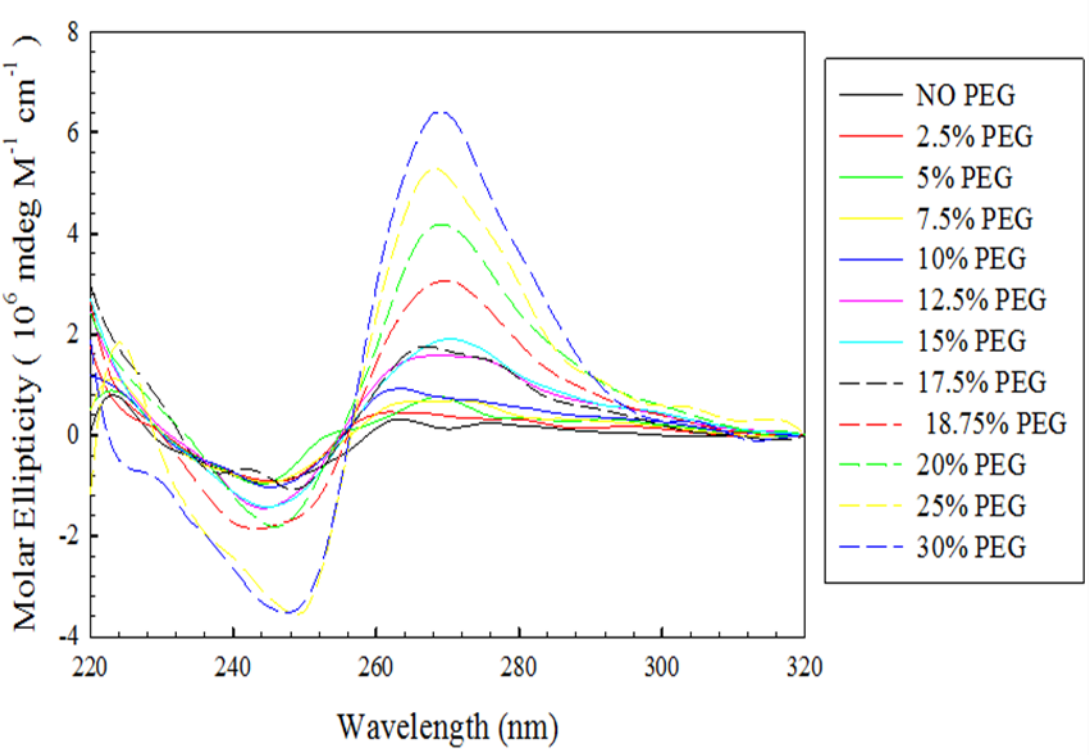


Figure 3.12: CD spectra of $(TTAGGG)_4$ at 95 ^{0}C. Spectra were taken in standard potassium phosphate buffer (115 mM K^{+}) as a function of percent weight PEG 6000.

Since there are two possible conformations of quadruplexes under different conditions of solutions, there would be two T_m values. To determine the T_m values of hybrid quadruplexes, the melting profiles of HTEL1 in solutions with no PEG, 5% PEG, and 10% PEG were plotted as displayed in Figure 3.13. The values of T_m were determined approximately as 68 ^{o}C, 74 ^{o}C, and 80 ^{o}C in no PEG, 5% PEG, and 10% PEG solutions

respectively. The T_m values of the hybrid quadruplexes in solutions with more than 10% PEG were not determine as the intensity of the peak at 292 nm was decreased significantly. However, it is safe to assume that hybrid quadruplexes formed in solutions containing more than 10% PEG would have higher T_m values.

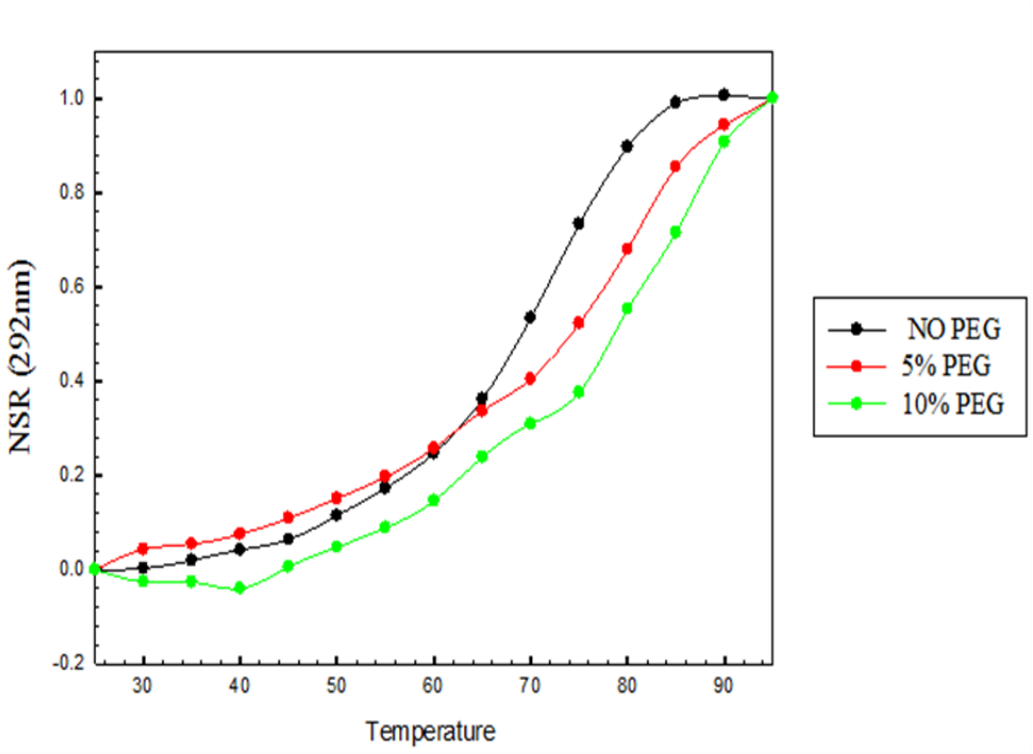


Figure 3.13: Melting profile of $(TTAGGG)_4$ in 0%, 5%, and 10% PEG solutions.

In addition, as the intensity of the peak at 270 nm was increased in solutions with more than 10% PEG, at the same time, the newly form parallel quadruplexes were not melted into single strands even at the highest possible temperature 95 ^{o}C. As a result, the T_m

values of quadruplexes of parallel conformation in solutions greater than 10% PEG could not be reasonably determined. For a long time, many researchers including us had thought that PEG is good chemical to assimilate cellular conditions. However, a recently published paper ruled out all the possibility of PEG as crowding agent[21]. Instead, PEG works on the basis of excluded volume effect and thus, not a good chemical to create cell conditions. As a result, we decided to compare results of PEG with the results of other chemicals with known properties. Acetonitrile is a known water disruptor and thus creates high osmotic pressure at high concentrations. In addition effect of trifluoroethanol on the quadruplex was also determined.

Effect of acetonitrile on conformations and stabilities

Figure 3.14 displays the CD spectra of HTEL1 at 25 ^{o}C at in standard phosphate buffer solution of 115 mM K^{+} as a function of percent volume of acetonitrile. As shown in the figure, the initial CD spectrum of HTEL1 in standard buffer solution contained a hybrid conformation with a peak at 292 nm and a shoulder at 270 nm. As the concentration of acetonitrile increases, the intensity of the peak at 292 nm diminishes and intensity of the shoulder at 270 nm is augmented. At about 40% acetonitrile solution we observe a very intense peak at 270 nm and minor shoulder at 272 nm indicating a complete transformation of the conformations from hybrid to parallel. A very similar trend was also observed in the CD spectra of HTEL1 in presence of PEG. A well-defined

isoelliptic point was also observed at about 282 nm during the transformation of conformations similar to one observed in case of PEG solutions.

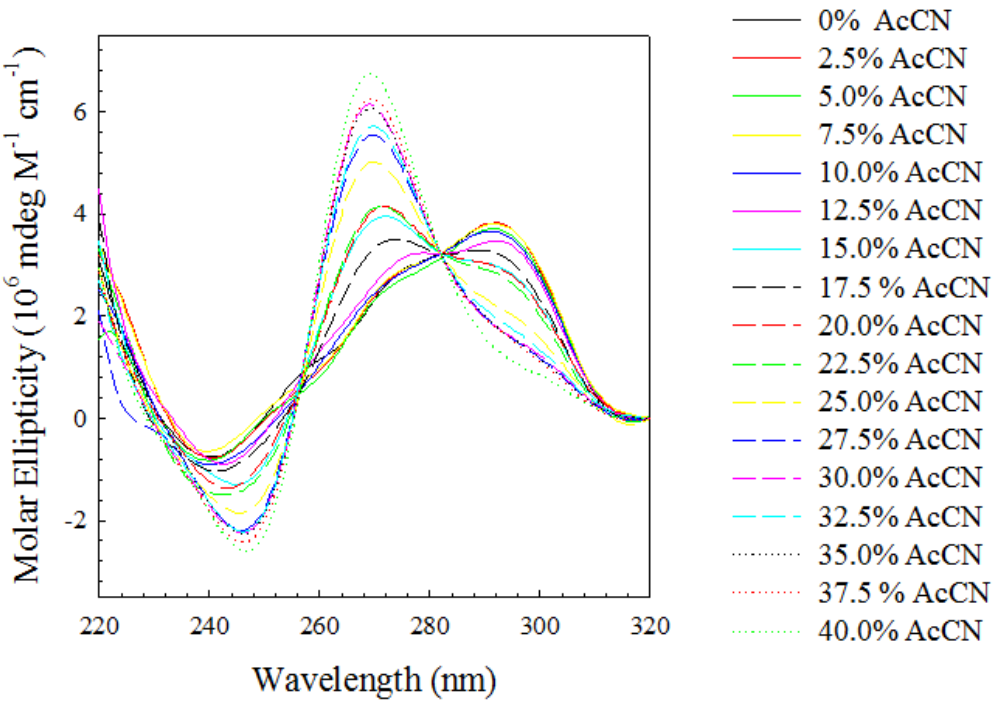


Figure 3.14: CD spectra of $(TTAGGG)_4$ in acetonitrile. Spectra were taken in standard phosphate buffer (pH 7.0, 115 mM K^+) at 25 ^{0}C in the absence and presence of increasing weight percent of acetonitrile.

In order to compare the stability of the quadruplexes formed in different acetonitrile solutions, CD optical melting studies of HTEL1 were done in standard phosphate solution as a function of percent volume acetonitrile. The melting spectra of 10%, 20%, 30% and 40% acetonitrile solutions are shown in Figure 3.15. The CD spectra of all

samples were taken in a temperature range of 25 ^{o}C to 95 ^{o}C with an increment of 5 ^{o}C and an incubation period of 5 minutes.

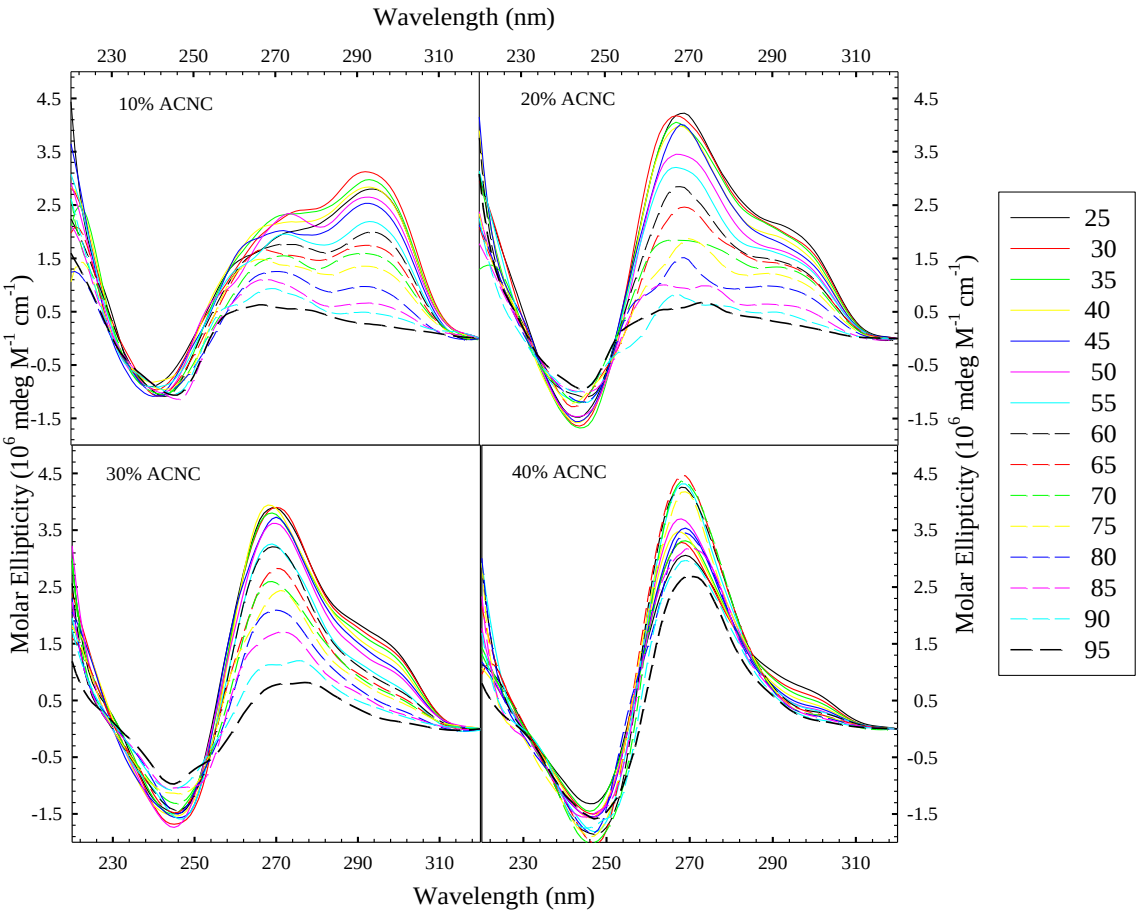


Figure 3.15: Typical CD optical melting spectra of $(TTAGGG)_4$ in acetonitrile. Spectra were taken in standard potassium phosphate buffer (115 mM K^+) as a function of acetonitrile concentration.

Figure 3.15 displays the CD spectra of HTEL1 as a function of temperature with different % acetonitrile. As the concentration of acetonitrile is increased, the stability of parallel conformational quadruplex is also increased.

As the temperature increases, the intensities of the peaks at 292 nm and 270 nm gradually decrease. A very similar trend of the same sequence was also observed in PEG solutions of different concentrations. However, extend of stability of the quadruplex formed in acetonitrile solutions was different as that of quadruplex formed in PEG solutions. For example, the scan of HTEL1 at 95 ^{o}C in 20% PEG solution had a significant intensity in the peak at 270 nm, whereas, the same spectrum in 20% acetonitrile solutions barely had any intensity in the peak at 270 nm. Though, the same peak became prominent in 40% acetonitrile solution. In comparison, the stability of quadruplexes formed by HTEL1 sequence in 20% PEG solution is approximately equivalent as that of quadruplexes in 40% acetonitrile solutions.

Effect of trifluoroethanol on conformations

The equilibrium studies of the two conformations were also studied in trifluoroethanol. The CD spectra of HTEL1 at 25 ^{o}C were recorded in various concentrations of trifluoroethanol as displayed in Figure 3.16. As can be seen in the figure, a gradual transition is observed from the normal spectrum to the parallel spectrum. As the concentration of trifluoroethanol increases, the peak at 292 nm slowly decreases and the peak at 272 nm slowly increases. However, unlike the CD spectra of HTEL1 in

PEG 6000 and acetonitrile, the transformation of the conformations was very slow as the concentration of the trifluoroethanol was increased. In addition, a complete transformation from hybrid to parallel conformations was not accomplished even at 40% trifluoroethanol. The CD spectrum of HTEL1 in 40% trifluoroethanol was similar to the CD spectra of HTEL1 observed in 15% PEG and 22.5% acetonitrile solutions.

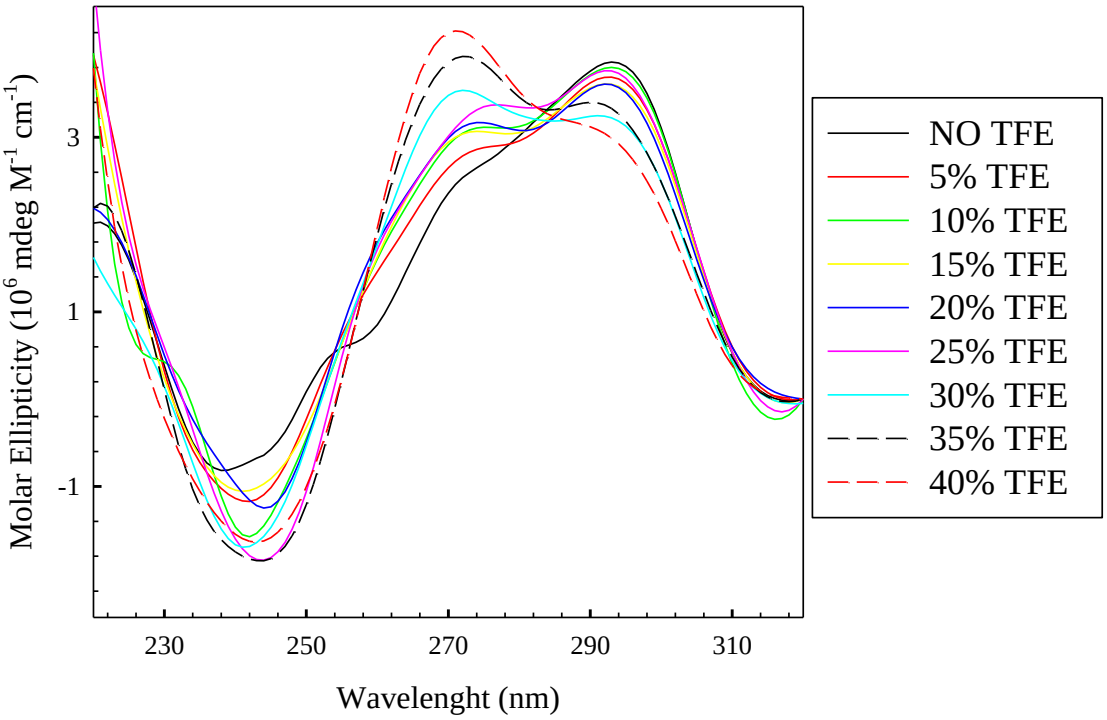


Figure 3.16: CD spectra of $(TTAGGG)_4$ in trifluoroethanol. Spectra were taken in standard phosphate buffer (pH 7.0, 115 mM K^+) at 25 ^oC in the absence and presence of increasing volume percent of trifluoroethanol.

Betaine as an osmolyte

An attempt to make 20% betaine solution in standard phosphate buffer at pH 7 and 115 mM K^+ was a fail. Addition of betaine in phosphate buffer dropped the pH of the solution to 0.5 and the solution, in addition, acted as a buffer at low pH. In order to raise the pH back to 7, potassium hydroxide (KOH) was added and as a result it increased the concentration of K^+ ion from 115 mM. Furthermore, it also diluted the solution and decreased the percent betaine in the solution. By the time pH reached at 7 from 0.5, the percent betaine in the solution dropped to 0.5% from 20%. As a result, we could not use betaine as an osmolyte.

Comparison of osmolytes

The presence of the any above osmolytes drives the equilibrium from hybrid conformation to parallel conformation of the quadruplex. However, the degree of change in conformations was different for different osmolytes. The transformation of conformation was most completed in PEG 6000 and least completed in trifluoroethanol. As a result, we decided to compare the osmotic pressure of osmolytes with each other. A plot of osmotic pressure vs. % cosolutes (PEG 6000, acetonitrile, and trifluoroethanol) is displayed in Figure 3.17.

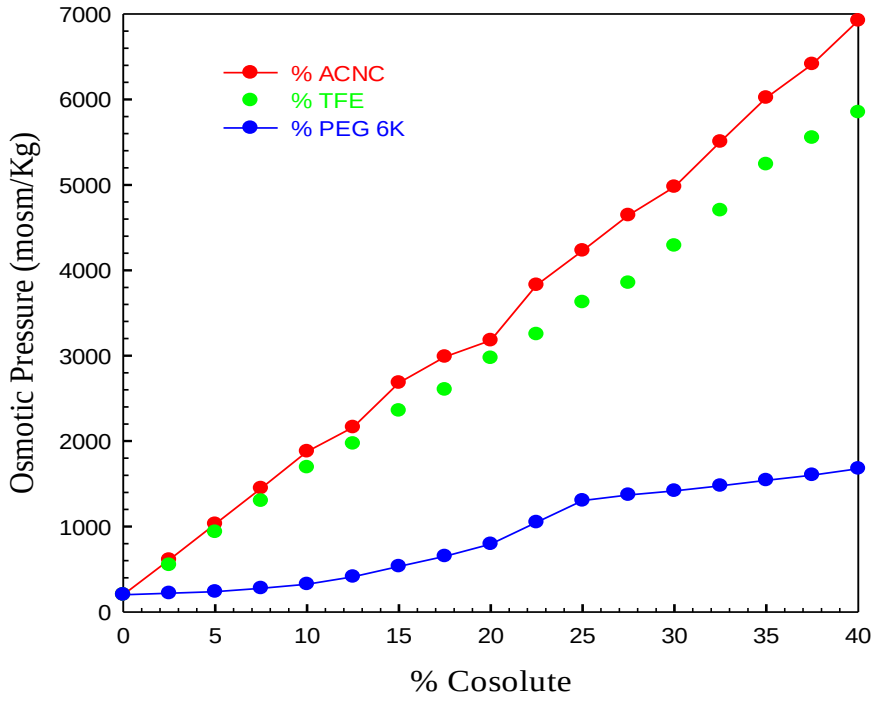


Figure 3.17: A comparison of the percent cosolute to osmotic pressure. Spectra for acetonitrile (%V/V), trifluoroethanol (%V/V) and PEG 6,000 (%W/V) were taken in standard phosphate buffer, pH 7.0, 115 mM K^+.

Osmolality is directly proportional to $\ln(\alpha_w)$, where α_w is the activity of water. Acetonitrile and trifluoroethanol are clearly more effective in disrupting the water activity than PEG 6000. In addition, the behavior of acetonitrile and trifluoroethanol are linear, whereas the behavior of PEG 6,000 is not so linear. This deviation of PEG from linearity might be due to number of factors including association with the DNA conformation as well as self-aggregation

Possibility of self-assembly

The conformations formed by $(TTAGGG)_4$ in 20% PEG and 40% acetonitrile were compared with the parallel quadruplex formed in the presence of Mg^{2+} in tris-borate buffer(23). The CD spectra of oligomer $(TTAGGG)_4$ at 25 ^{o}C in 10 mM TBE solution of pH 8.0, 115 mM K^+, and 20 mM Mg^{2+} was also recorded. Figure 3.18 compares the CD spectra of HTEL1 under five different conditions: (1) phosphate buffer, pH 7.0 with 115 mM Na^+ (pink); (2) phosphate buffer, pH 7.0 with 115 mM K^+(black); (3) 10 mM TBE, pH 8.0 with 115 mM K^+ and 20 mM Mg^{2+} (blue); (4) phosphate buffer, pH 7.0 with 115 mM K^+ and 20% PEG (red); and (5) phosphate buffer, pH 7.0 with 115 mM K^+ and 40% acetonitrile (green)

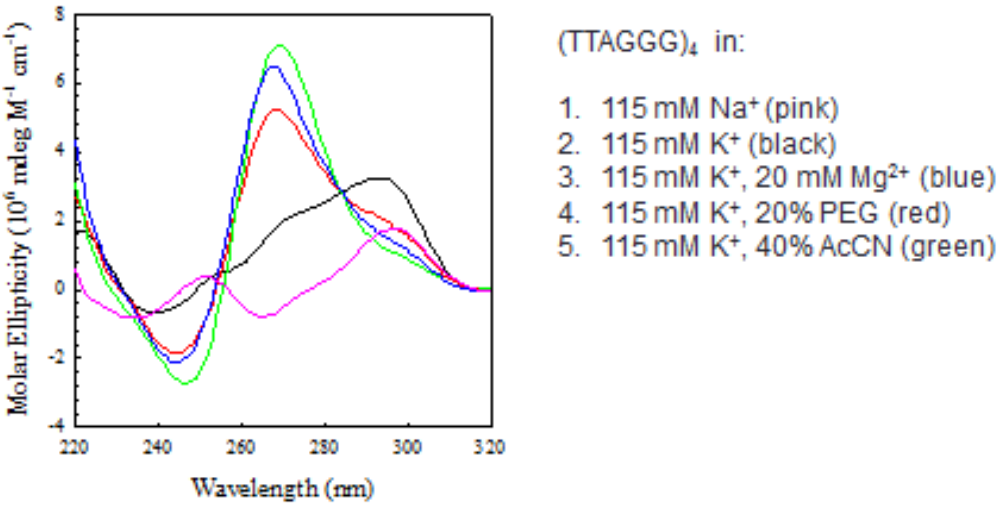
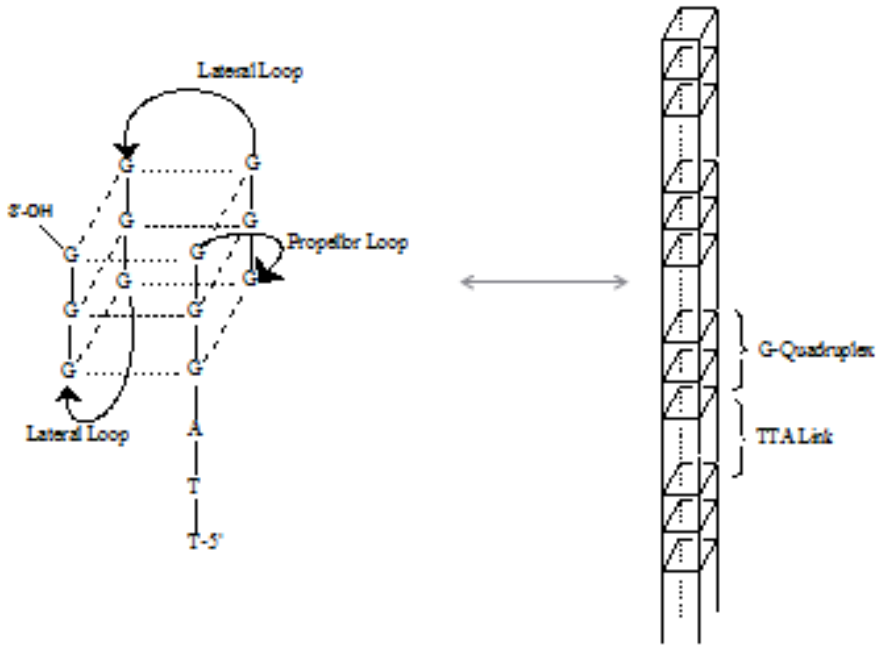


Figure 3.18: CD spectra of $(TTAGGG)_4$ under five different conditions.

Previously, the self-assembly of DNA oligomer of sequence $C_4T_4G_4T_{1-4}G_4$ into a conformation of high molecular weight in the presence of Mg^{2+} had been reported[22-24] and CD spectra of this particular sequence is very similar to the CD spectra of $(TTAGGG)_4$ in the same conditions. On the other hand, in the absence of Mg^{2+}, the unimolecular hairpin structure was observed. As a result, we could not rule out the possibility of the self-assembly of $(TTAGGG)_4$ oligomer in 10 mM TBE solution of pH 8.0, 115 mM K^+, and 20 mM Mg^{2+} . Three spectra of HTEL1 in TBE Mg^{2+}, PEG 20%, and 40% acetonitrile solutions as displayed in Figure 3.18 are very similar to each other. Thus, the presence of same higher order structure in 20% PEG and 40% acetonitrile solutions is also highly likely. To explain this, we suggest two schemes on the transition from a unimolecular quadruplex into a multimolecular quadruplex

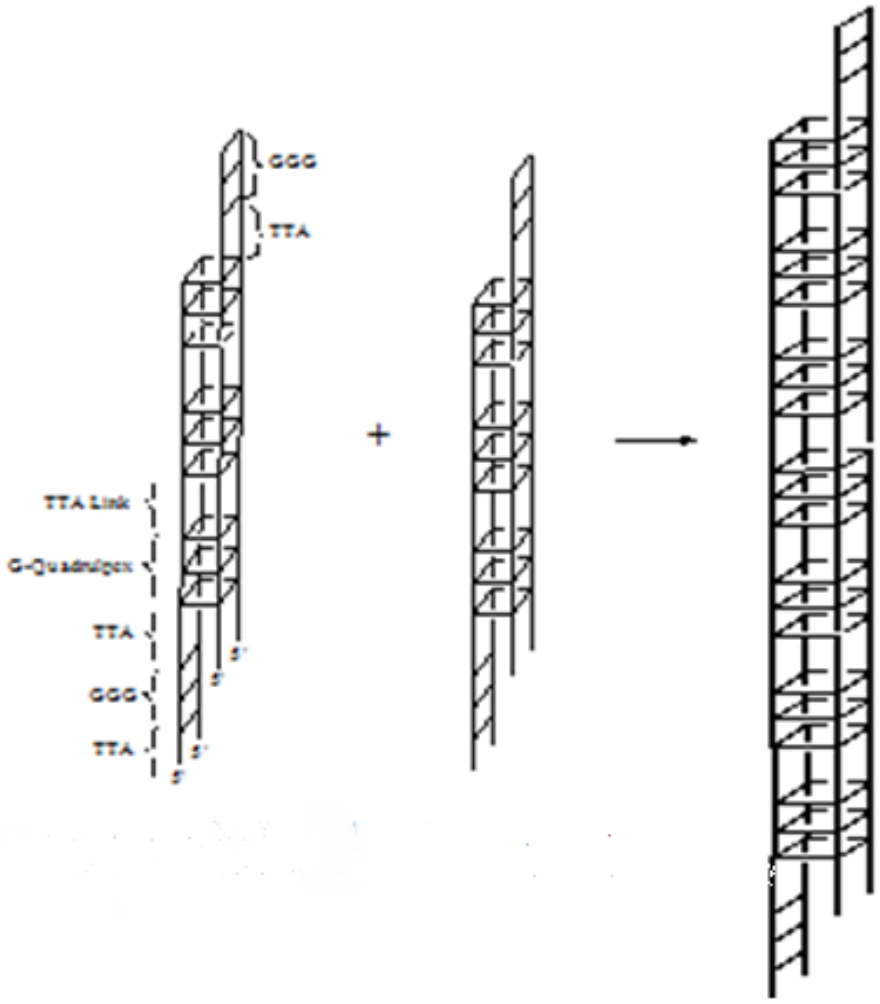
Scheme 1 shows (left) the unimolecular conformation that contains two lateral and one propeller loop which is found in the presence of K^+ and is converted to a parallel stranded quadruplex with a molecularity of four. In this case, four strands of $(TTAGGG)_4$ join with each other in such a way that the first set of G-bases of all four strands are in register with each other and, as a result, yields a blunt ended structure. Since the intensity of the peak at 270 nm in 40% acetonitrile solution is greater than the intensity in Mg^{2+} TBE solution, a self-assembled structure with molecularity more than four is also possible..

Scheme 1: The transition of conformations. Scheme shows a transition from a unimolecular conformation to a termolecular conformation.



In this situation, we could have two strands out of register with respect to other two strands. This particular association will give them sticky ends and similar structures could connect each other and make structures with a molecularity of more than four as shown in scheme 2.

Scheme 2: The self-assembly of $(TTAGGG)_4$.



As a result, there is high probability of a self-assemble mechanism and, as such, we cannot rule out the possibility of the structures with molecularities of more than four. However, further studies needs to be done with the help of NMR or gel electrophoresis to confirm these possibilities.

CD studies of the duplex

The CD melting studies of the duplex formed after mixing a 1:1 mole ratio of $(TTAGGG)_4$ and $(CCCTAA)_4$ were also determined in the presence of PEG 6000. The concentrations of $(TTAGGG)_4$ and $(CCCTAA)_4$ were determined very carefully and their calculated volumes were mixed extremely cautiously.

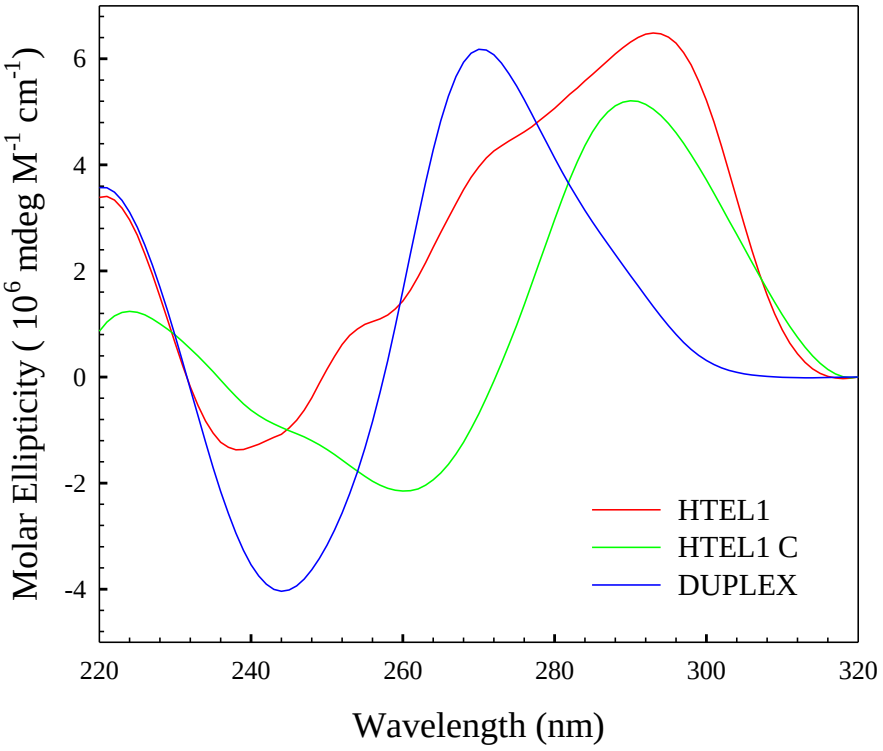


Figure 3.19: CD spectra of $(TTAGGG)_4$, $(CCCTAA)_4$, and duplex. Spectra were taken at 25 ^oC in standard phosphate at pH 7.0, 115 mM K^+.

Figure 3.19 displays the CD spectra of $(TTAGGG)_4$ and $(CCCTAA)_4$ in phosphate buffer, pH 7.0 at 115 mM K^+ before mixing with each other and spectrum of duplex is also shown in the same figure. The CD spectra of $(TTAGGG)_4$ and $(CCCTAA)_4$ contain major peaks at 292 nm and 285 nm which represent the conformations of hybrid quadruplex and single strand respectively. When the two sequences were mixed together, the CD spectrum of the resultant shows a major peak and a trough at about 270 nm and 242nm respectively which represent double stranded DNA structure. It has been found that the quadruplex structures are present not only at the telomeric regions but also in the middle of the chromosomes[25]. We tried to test if PEG can induce the formation of the quadruplexes from double stranded DNA structure. However, even after adding different percentages of the PEG in the solution, it didn't change double stranded to quadruplex.

The CD optical melting studies were also carried out for duplex as a function of % PEG 6000. Figure 3.20 displays the CD spectra of duplex as a function of temperature with different PEG 6000 concentration. As the temperature increases, the intensity of the peak at 270 nm decreases. However, the CD scans at the 95 ^oC for duplex in no PEG, 10% PEG, and 20% PEG solution still showed a significant intensity of the peak at 270 nm indicating that the duplex structure was present at the higher temperatures.

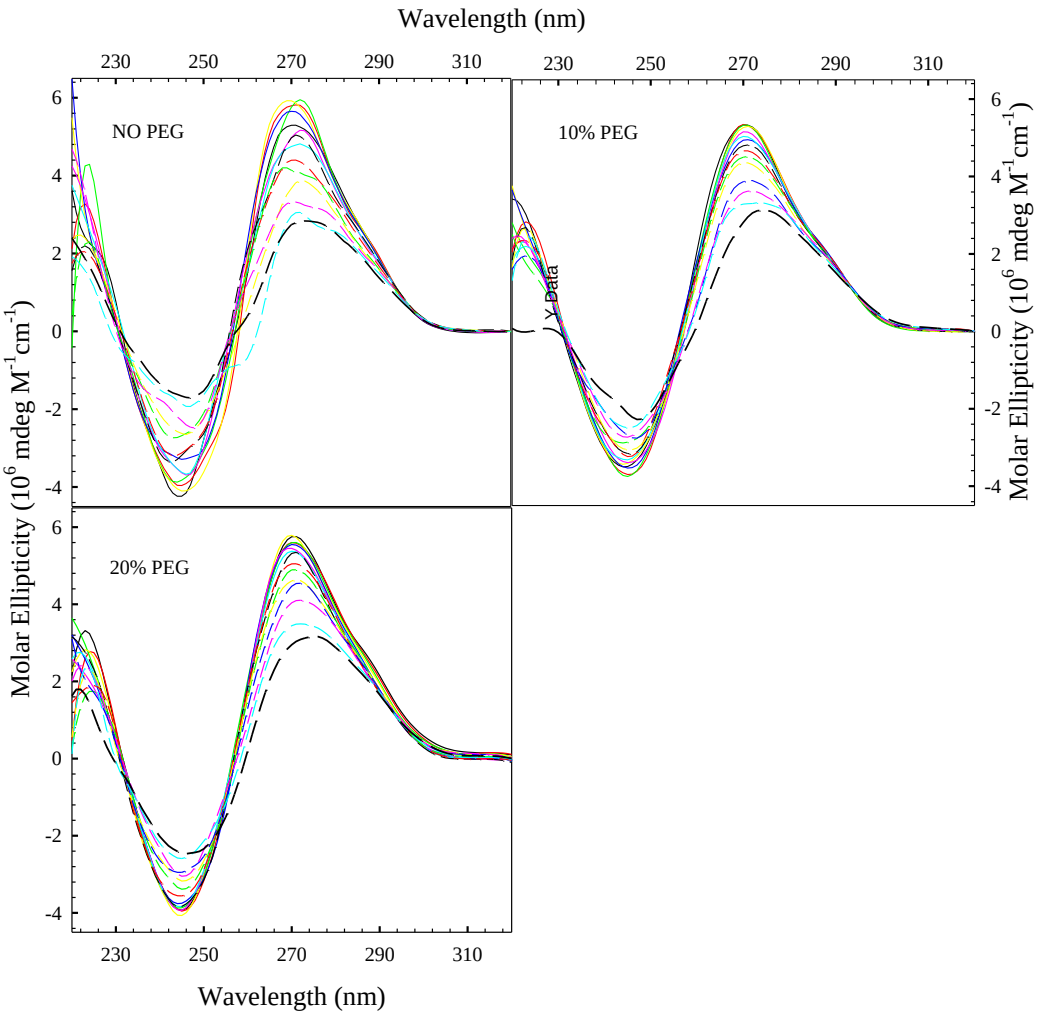


Figure 3.20: CD optical melting spectra of duplex. Spectra were taken in standard phosphate at pH 7.0, 115 mM K^+ as a function of PEG.

The intensity of the peak at the highest temperature was increased as the % of PEG was increased. This implies that the stability of the duplex is increased as PEG percentage is raised. We have found that in the absence of PEG, $(TTAGGG)_4$ undergoes a transition from the fully folded state at 25 ^oC to a fully unfolded state at 95 ^oC. However, in the

absence of PEG, the duplex did not undergo a complete transition from fully folded to fully unfolded state. This also confirms that there are interactions other than just H-bonds in duplex conformation that provide extra stability to the structure.

A DSC attempt to obtain a value of T_m

Differential Scanning Calorimetry (DSC) is used to determine T_m value of HTEL1 in 10% PEG solution.

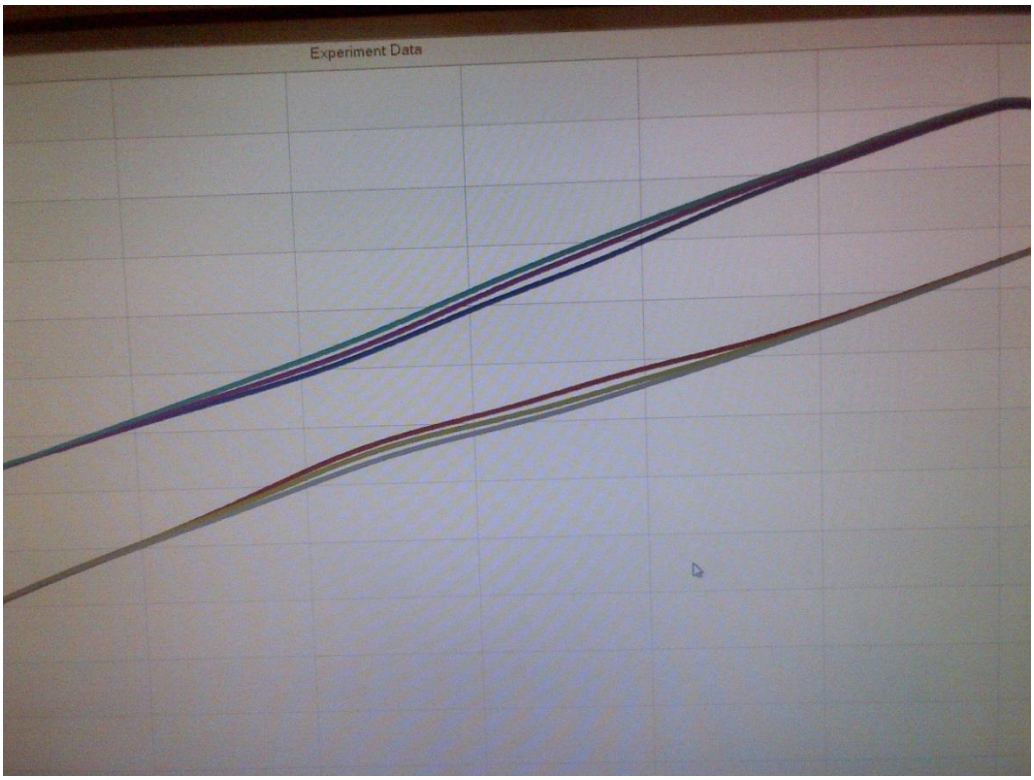


Figure 3.21: DSC heating and cooling spectra of $(TTAGGG)_4$. Spectra were taken in standard phosphate at pH 7.0, 115 mM K^+ and 10% PEG 6000 as a function of temperature.

The heating and cooling scans of the sample from DSC are shown in Figure 3.21 and heating scans are not superimposed on each other. As a result, valuable information about T_m could not be obtained. The experiment was repeated multiple times in different conditions; however, the non-superimposed scans were obtained every time.

CHAPTER IV

SUMMARY AND CONCLUSION

The conformational properties of G-quadruplexes formed by human telomere sequence are very sensitive to environmental conditions. In standard phosphate buffer with 115 mM K^+, quadruplex with hybrid unimolecular conformation is observed. As the concentration of different osmolytes (PEG, Trifluoroethanol, and Acetonitrile) is slightly increased, a gradual change in the conformation of quadruplex from hybrid to parallel structure is observed. However, pace of transformation of conformation of quadruplex is different in various osmolytes. In addition, a complete transformation is not achieved as the concentration of trifluoroethanol is increased in the solution. The resultant CD spectra are similar to that of the sequence in the presence of Mg^{2+} - a known inducer of G-wire formation. The stability of the quadruplex formed in different osmolytes is different as quadruplex formed in the presence of PEG is most stable than formed in presence of acetonitrile with the same concentration.

The mechanism of transformation of conformation is different in different osmolytes. The PEG works on excluded volume mechanism and preferentially binds to parallel conformation of quadruplex and as a result, its presence drives the equilibrium from unimolecular hybrid to multimolecular parallel conformation[21]. On the other hand,

in case of acetonitrile and trifluoroethanol, the conformational changes are due to changes in water activity and how water interacts with the DNA backbone and bases.

REFERENCES

1) The Nobel Prize in Physiology or Medicine 2009 Elizabeth H. Blackburn, Carol W. Greider, Jack W. Szostak

2) Watson, J. D., Crick, F. H. C. Molecular structure of nucleic acids – a structure for deoxyribose nucleic acid. Nature 1953, 171, 737-738.

3) Balagurumoorthy, P.; Brahmachari, S. K.; Mohanty, D.; Bansal, M.; Sassisekharan, V. "Hairpin and Parallel Quartet Structure for Telomeric Sequences." Nucleic Acids Research 20, 4061-4067 (1992).

4) Formation of a Stable Triplex from a Single DNA Strand Sklenar, Vladimir;Feigon, Juli Nature;Jun 28, 1990;345,6278;ProQuest Nursing & Allied Health Source pg. 836

5) Venczel, E.A., Sen, D. Parallel and antiparallel G-DNA structures from a complex telomeric sequence. Biochemistry 1993, 32, 6220-6228.

6) Williamson. J.R. G-Tetrad structures in telomeric DNA. Annual Reviews in Biophysical and Biomolecular Structure 1994, 23, 703-730.

7) Luu, K.N., Phan, A.T., Kuryavyi, V., Lacroix, L., Patel, D. Structure of the human telomere in K+ solution: an intramolecular (3+1) G-quadruplex scaffold. Journal of the American Chemical Society 2006, 128, 9963-9970.

8) Wang, Y., Patel, D.J. Solution structure of the human telomeric repeat d[AG3(T2AG3)3] G-tetraplex. Structure 1993, 1, 263-282.

9) Burge, S., Parkinson, G. N., Hazel, P., Todd, A. K., Neidle, S. Quadruplex DNA: sequence, topology and structure. Nucleic Acids Research 2006, 34, 5402-5415.

10) Ambrus, A., Chen, D., Dai, J., Bialis, T., Jones, R., Yang, D. Human telomeric sequence forms a hybrid-type intramolecular G-quadruplex with mixed parallel/antiparallel strands in potassium solution. Nucleic Acids Research 2006, 34, 2723-2735.

11) A theory of marginotomy: The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. Journal of theoretical biology [0022-5193] Olovnikov, A M yr:1973 vol:41 iss:1 pg:181 - 190

12) The limited in vitro lifetime of human diploid cell strains Hayflick, L. Experimental Cell Research, 1965, Vol.37(3).

13) Miyoshi, D., Nakao, A., Sugimoto, N. Molecular crowding regulates the structural switch of the DNA G-quadruplex. Biochemistry 2002, 41, 15017-15024.

14) Xue, Y., Kan, Z.-Y., Wang, Q., Yao, Y., Liu, J., Hao, Y.-H., Tan, Z. Human telomeric DNA form parallel-stranded intramolecular G-quadruplex in K+ solution under molecular crowding condition. Journal of the American Chemical Society 2007, 129, 11185-11191

15) Heddi, B., Phan, A. T. Structure of human telomeric DNA in crowded solution. Journal of the American Chemical Society 2011, 133, 9824-9833.

16) Sun, D., Hurley, L.H. Targeting telomers and telomerase. Methods in Enzymology, 2001, 340, 573 -592.

17) Han, H., Hurley, L.H. G-quadruplex DNA: A potential target for anti-cancer drug design. TiPS 2000, 21, 136-142.

18) Jenkins, T.C. Targeting multi-stranded DNA structures. Current Medicinal Chemistry 2000, 7, 99-115.

19) Neidle, S., Read, M.A. G-quadruplexes as therapeutic agents. Biopolymers 2001, 56, 195-208.

20) Wong, H. M., Payet, L., Huppert, J. L. Function and targeting of G-quadruplexes. Current Opinion in Molecular Therapeutics 2009, 11, 146-155.

21) Buscaglia, R.; Miller, M. C.; Dean, W.L.; Gray, R. D.; Lane, A.N.; Trent, J.O.; Chaires, J.B. Polyethylene glycol binding alters human telomere G-quadruplex structure by conformational selection. Nucleic acid. Res. 2013, 41, 7934-7946.

22) Sen, D.; Gilbert, W. Superstructures formed by telomere like oligomers. Biochemistry 1992, 31, 65-70.

23) Marsh, T.C.; Henderson, E. G-Wires: Self-assembly of a telomeric oligonucleotide, d(GGGTTGGG), into large superstructures. Biochemistry 1994, 33, 10718-10724.

24) Shi, Y.; Luo Q.; Li, N.B. A highly sensitive resonance Rayleigh scattering method to discriminate a parallel-stranded G-quadruplex from DNA with other topologies and structures. Chem. Commun. 2013, 49, 6209-6211.

25) Katapadi VK1, Nambiar M, Raghavan SC. Potential G-quadruplex formation at breakpoint regions of chromosomal translocations in cancer may explain their fragility. 2012 Aug;100(2):72-80. doi: 10.1016/j.ygeno.2012.05.008