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INTRODUCTION
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1.0 Introduction

DNA from the telomeres contains a stretch of simple randomly repeated sequences in which clusters of G residues alternate with clusters of T/A sequences along one DNA strand. Model telomeric G clusters form four stranded structures in the presence of Na(I) K(I) Sr(II) Ba(II) Ca(II) Lu(I) and NH₄(I) ions. Telomeres are specialized DNA protein structures at the termini of chromosomes which have been shown to be important for their stability and accurate replication. Telomeric DNA of eukaryotes consists of simple repetitive guanine-rich sequences having the general formula (T/A)₁₄G₁₈ [1, 2]. The telomeric G-rich single stranded DNA can adopt in vitro an intramolecular and intermolecular G quadruplex structure even at a very low NH₄(I) ion concentration (1 mM) [3]. Synthetic oligonucleotides containing one or more copies of G-rich telomeric sequences form defined structures in solution in the presence of Na(I) K(I) stabilized by Hoogsteen hydrogen bonding between four G bases [4, 8]. G quadruplexes are the most recent secondary DNA structures being considered as targets for drug design in cancer therapy because it has been shown to directly inhibit telomerase elongation in vitro [9]. Telomerase is expressed in tumor cells but not in most of the somatic cells. Therefore, a drug or any molecule that stabilizes quadruplex structure could interfere with telomere elongation and replication.
of cancer cells. Hence, it has been suggested that studies on G quadruplexes stabilization and destabilization might be appropriate for the cancer therapy.

Earlier studies also show that the transformations of normal human cells to tumor cells have established an intimate link between tumor immortalization and activity of the enzyme telomerase [10, 11]. Telomerase has emerged as an obligatory anti-cancer chemotherapeutic target [12, 13] and considerable effort is now focused on the design of agents with inhibitory activity. Earlier reports [14, 15] indicate that G-rich DNA sequences of this type can be assembled to form four-stranded G quadruplex structures which can be targeted to effect an indirect inhibition of the enzyme namely telomerase. Keeping the importance of studies on the state of G quadruplex DNA structure which has influence on the activity of telomerase, studies were performed in this part of the work where drugs and metal ions show influence on the state and stability of G quadruplex structure when they were allowed to interact with the synthetic DNA in vitro.

The structure-function relationship of telomeres is of immense interest in recent times as they offer understanding of few unique biological processes. It has been reported earlier that telomeres are stabilized by G quadruplex formation where the internal cavity of the tetrad is occupied by a monovalent cation [16]. Thermodynamic stability of the G tetrad structures in the presence of various cations are usually measured by the temperature dependence of the typical circular dichroism bands of the G tetrad or by thermal melting studies of the DNA [17]. It has been reported earlier that [18]
Tb(III) induced luminescence of the telomeric structures is also a good measure of its stability. It has been demonstrated that [19] a divalent cation Sr(II) facilitates intermolecular G quadruplex formation at lower concentration in comparison to that required by K(I) ion for similar effect. Telomeric structures have the potential to form both inter and intramolecular G quadruplex which can be analyzed by their differential electrophoretic mobilities after thermal treatment [19]. For the past several years, it was the area of interest to find out whether monovalent cations respond to preferential stabilization of inter- or intramolecular tetrad structures. The answer to such a problem may lead to better understanding of structure-function relationship in telomeric ends. The fact that DNA and RNA can form helices containing more than two strands has been known since the earliest studies of nucleic acid structure. The past decade has seen resurgence of interest in two separate lines of investigations that the potential use of nucleic acids as therapeutic [20, 22] and evidence that alternative (i.e., non-B DNA) structure may have specific functional rules in vivo [23, 24].

1.1 Guanine Tetrads

Gellert et al. have proposed that guanosine and its derivatives at millimolar concentration and above form viscous gels in water but it was not until the first x-ray diffraction data was available some fifty years later that this peculiar behavior could be explained [25]. Tetrameric arrangement of the guanine bases in guanosine 5-monophosphate gels and a structure termed as the guanine tetrad, also known as guanine quartet, simply G quartet or G
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quadruplex have proposed as a molecular basis for the gel formation phenomenon The guanine tetrad is a square co planar array of four guanine bases in which each base is both the donor and acceptor of two hydrogen bonds with its neighbors More precisely the guanine tetrad arises from the association of four guanosine into a cyclic Hoogesteen hydrogen bonding arrangement that involves N1 N7 O6 and N2 of each guanine base (Figure 1) Detailed biophysical studies on guanosine 3 phosphate by Sasishekaran et al showed that this molecule aggregated into guanine tetrad structures [26] Subsequent examination of more than 60 guanine derivatives with either ribose or deoxyribose sugar moieties by Guschlbauer et al has confirmed the original proposal of the guanine tetrad arrangement [27]

The synthesis of poly (G) homopolymers was achieved considerably later than that of all other polynucleotides and Pochon and Michelson have found to form a strong self structure [28] It would not melt even in the absence of ions and interactions with complementary poly (C) homopolymers were poor Circular dichroism [29] by Thule and Guschlbauer and infrared spectroscopy (30) by Howard et al resulted in spectra similar to those of guanosine gels X ray fiber diffraction studies of poly (G) homopolymers established the existence of a four stranded nucleic acid structure with stacked guanine tetrads [31 33] The poly (dG) homopolymer was initially even less well characterized than poly (G) mainly because of difficulties in obtaining suitable material for experimentation
Figure 1 The guanine tetrad motif and its hydrogen bonding scheme (left) and a space filling model revealing the hollow centre (right)
Today custom DNA synthesis of oligonucleotides with stretches of consecutive guanines still result in substantially lower yields. However Lefler and Bollum found that poly (dG) could be synthesized by terminal deoxynucleotidyl transferase [34] which enabled characterization of poly (dG) by circular dichroism [35]. Even though it could not be confirmed by X-ray fiber diffraction the poly (dG) homopolymer was concluded to be four stranded.

Little attention was paid to the phenomenon of guanine tetrads and for more than 25 years it remained a structure in search of function. Then suddenly it was implicated in various events of almost biological significance. Sen and Gilbert suggested that guanine tetrads were responsible for the switch recombination that immunoglobulin heavy chains undergo to bring different constant regions next to variable regions during the differentiation of B lymphocytes [5]. G quadruplexes were also shown to form at the telomeric ends of eukaryotic chromosomes [48] in the promoter regions of oncogenes [36–38] and Hammond Kosack et al. showed that it is involved in the regulation of the insulin gene [39].

1.2 G quadruplexes

In DNA two or more guanine tetrads can stack upon each to form four stranded structures with a guanine tetrad core. They are collectively referred to as G quadruplexes or DNA tetraplexes. The term G quadruplex refers to any four stranded DNA structure containing guanine tetrads without reference.
to strand connectivity. Despite a unifying origin, the most intriguing aspect of G quadruplexes is their extensive polymorphism, which can be divided into a number of subclasses. For pedagogic purposes, they will be treated as isolated phenomena here, but it is evident that the possible numbers of conformations a given sequence can adopt are sometimes restricted by mutually exclusive subclasses of polymorphism.

1.3 Variations on the G quadruplex Theme

1.3 (a) Strand Stoichiometry

Strand stoichiometry variation allows G quadruplexes to be formed by association of one strand [4 40 41] as shown in Figure 2, two stranded [8 42] as shown in Figure 3 or four stranded [5 43] as in Figure 4. In principle, three stranded arrangements are conceivable but have yet to be substantiated. Moreover, if one strand has the ability to form a unimolecular structure, it could also form bimolecular or quadrimeolecular structure [17 44 46]. What determines a priority with which these structures form depends on strand concentration.

1.3 (b) Strand Polarity Polymorphism

The next fundamental source of structural variation is the relative arrangement of adjacent backbones, which can have different polarities. Irrespective of whether they are part of the same molecule or not, the strand or strands that constitute a G quadruplex can come together in four different ways. They can be all parallel as evident from Figure 5A.
Various strand stoichiometries of G quadruplex structures

Figure 2  One stranded structure yields a unimolecular G quadruplex

Figure 3  Two strands render a bimolecular G quadruplex

Figure 4  Four separate strands produce a quadrimolecular G quadruplex
one antiparallel as shown in Figure 5B adjacent parallel like Figure 5C or alternating antiparallel as shown in Figure 5D. Strand polarity configurations have been determined for various sequences. Many short oligonucleotides which guanine tracts have been found either by NMR spectroscopy or by crystallography [47, 49] can adopt G quadruplex structures in which all strands are parallel. NMR spectroscopy structure determinations of the Tetrahymena [50] and Oxytricha [41] telomere repeat sequences are examples of G quadruplex structures that have three parallel strands and one antiparallel strand. There are many examples of G quadruplex structures that have two pairs of adjacent parallel strands [51, 53] as well as alternating antiparallel strands [54, 57].

13 (c) Glycosidic Torsion Angle Variation

The bases in normal B DNA are found only in the anti conformation whereas guanines involved in formation of guanine tetrads are observed both in syn and anti conformations (Figure 6). However restrictions apply to adjacent guanines involved in the same guanine tetrad. If they are on parallel strands, they must have the same glycosidic torsion angles and conversely if they are on antiparallel strands, they must have opposite glycosidic torsion angles. The glycosidic conformation changes the relative orientations of the bases in contiguous guanine tetrads and affects the stacking energy. While normal B DNA has one minor and one major groove, the stacking of guanine tetrads produces four grooves that are not necessarily identical but can be wide, medium or narrow. If all four strands that participate in a guanine tetrad
Figure 5  Different strand polarity arrangements of G quadruplexes
(Arrows indicate 5' to 3' polarity)

5A  All strands parallel
5B  Three parallel strands and one strand antiparallel
5C  Two pairs of adjacent parallel strands
5D  Alternating antiparallel strands
core are parallel the four grooves are all of medium size. However, if the guanine tetrad core has antiparallel strands it can result in wide, medium, and narrow grooves. In the first case, for guanine tetrad core that is built from two pairs of adjacent parallel strands, guanines that belong to adjacent strands have the same glycosidic torsion angles produce medium grooves whereas guanines that belong to adjacent antiparallel strands and have opposite glycosidic torsion angles produce one wide and one narrow groove. In the second case, for a guanine tetrad core that is built from alternating antiparallel strands exclusively (Figure 7) two wide and two narrow grooves are produced. This phenomenon extends the polymorphism of G quadruplexes further since loops that connect adjacent strands may produce grooves of any size. Based on theoretical calculations, Mohanty and Bansal suggested that tracts of guanines favor formation of G quadruplexes with four parallel strands in which all guanines adopt the anti conformation and those alternating anti/syn arrangements were restricted to intramolecular G quadruplexes with antiparallel strands [58]. It is easily realized that any G quadruplex structure that contains antiparallel strands must have bases in both the anti and syn conformation in order to maintain the tetrad base pairing scheme. There are examples of virtually any combinations of glycosidic torsion angles. Several structures with exclusively anti [59] or syn [4] conformations have been characterized and structures with regularly alternating anti/syn conformations [17, 53, 54, 60, 61] or mixtures of anti/syn
Figure 6  Guanine quadruplex with two pairs of adjacent parallel strands that have identical glycosidic torsion angles

Figure 7  Guanine quadruplex with exclusively alternating antiparallel strands
conformations within both guanine tracts and guanine tetrads [62 63] is observed

1.3 (d) Connecting Loops

The loops that connect guanine tracts participating in the formation of unimolecular or bimolecular G quadruplexes can run in a number of different ways. The two strands involved in bimolecular G quadruplexes can have loops that connect guanine tracts either diagonally or edgewise. Diagonal loops are expected to protrude on opposite ends of the guanine tetrad core as shown in Figure 8A [57]. Although bimolecular G quadruplexes with two diagonal loops on the same side are conceivable, their formation is highly unlikely due to both steric hindrance and electrostatic repulsion between the two negatively charged backbones. If the two loops connect guanine tracts edgewise, they can protrude either on the same or on opposite sides of the tetrad core. Loops protruding on the same side of the core can be either parallel (Figure 8B) or antiparallel (Figure 8C). When the two loops protrude on opposite sides of the core, they can run in two different directions (Figures 8D and 8E).

For unimolecular G quadruplexes, the alternatives are probably fewer. In order to avoid the clash of two diagonal loops on the same side as described for bimolecular G quadruplexes above, the three loops can join either in the order adjacent adjacent adjacent (Figure 9A) or adjacent diagonal adjacent (Figure 9B). On the other hand, there is at least one example of parallel
Figure 8 Strand connectivity alternatives for bimolecular guanine quadruplex structures

8A Diagonal loops protruding on either side of the guanine quadruplex core
8B Two parallel edgewise loops protruding on the same side
8C Two antiparallel edgewise loops protruding on the same side
8D Adjacent parallel strands with edgewise loops protruding on opposite sides
8E Alternating antiparallel strands with edgewise loops protruding on opposite sides

Figure 9 Strand connectivity alternatives for unimolecular guanine quadruplex structure

9A All three loops run edgewise and connect adjacent adjacent adjacent adjacent
9B One diagonal and two edgewise loops that connect adjacent diagonal adjacent
9C A loop that runs on the outside of the guanine quadruplex core
strands connecting via loops running on the outside of the guanine tetrad core (Figure 9C) [50] which indicate that the spectra of the unimolecular structures may be more complex than prospected here

1 3 (e) Capping Bases/ Base Pairs/ Triads

From structural studies of various G quadruplexes it appears that most loops are not loose and flexible. For unpaired bases stacking interactions with the guanine tetrad closest to the loop are important. Any of the four bases forming the boundary that faces a guanine tetrad can engage in formation of planar structures that stack well on the tetrad core. Two such cases have been described so far. First, it has been found that d(TAGG), a truncated analog of the Bombyx mori telomeric repeat d(TTAGG) forms a four stranded G quadruplex in which the guanine tetrad core is sandwiched between two TAA triads [64] (Figure 10A). Second, it has been proposed that the formation of unimolecular G quadruplex structures just like hairpin or cruciform structures can bring together complementary bases that are located on the same strand and separated by a large number of bases. If the two complementary bases reside on antiparallel strands the guanine tetrad core can be capped by intrastrand base pairs of the normal Watson Crick type [37, 38] (Figure 10B)

1 3 (f) Inclusion of other Bases

The perfect sequence motif required for the formation of intrastand fold back G quadruplex DNA structures can be written $G_xN_{y_1}G_xN_{y_2}G_xN_{y_3}G_x$ where
Figure 10  Examples of how G quadruplex core structures can be capped

A  Two T A A triads (light gray) capy two planes of guanine tetrads (dark gray)

B  Two intrastrand AT base pairs (light grey) cap three planes of Guanine tetrads (dark gray) Unpaired bases (black) do not participate in formation of structures
x states the number of stacked guanine tetrads and y1 y2 and y3 dictates the loop lengths. However, a number of variations on this motif have been reported. Inclusion of adenine bases has been reported by Murchie and Ilalley for the human telomeric repeat d(TTAGGG)_n [65] (Figure 11). A number of base pairing schemes have been suggested but so far the details of the tetrads built from two adenines and two guanines remain to be elucidated.

Ashley and Warren have shown length polymorphism in d(CGG) tracts is associated with numerous diseases [66 67]. In vitro such sequences can adopt a G quadruplex structure with a core of guanine tetrads and looped out cystosines [68] (Figure 12). Under acidic conditions, the structure is further stabilized by formation of hemi protonated cytosine cytosine base pairs [69]. Another G quadruplex forming element in the chicken β globin promoter described earlier has been suggested to involve other bases than guanine in the core structure but no details are known [70].

13.3 Precise Coordination of Cations

The polymorphism exhibited by G quadruplexes is very much abundant than described so far. Perhaps the most interesting characteristic of these structures is their selective interaction with certain cations that fit well in the cavities formed by stacking of guanine tetrads. The cavity between two planes of guanine tetrads is lined by eight carbonyl oxygen 6 atoms that can all participate in the precise coordination of cations. The ability of potassium to stabilize guanine tetrads was first observed when it was found that the melting
Figure 11 Transformation of a G quadruplex into a structure that accommodates adenine bases (light grey) in the core structure

A When adenine bases are excluded from participation in quadruplex structure in human telomeric repeat d(TTAGGG)

B When adenine bases participate in the formation of quadruplex structure
Figure 12  Protonation Enables Cytosine$^+$ Cytosine Base Pairs (light gray) to stabilize G quadruplex structures (Protons are indicated as black spheres)

A Before protonation, the Cytosine$^+$ Cytosine base pairs are not participating in base pairing (non acidic conditions)

B After protonation, the quadruplex structure is stabilized by the formation of Cytosine$^+$ Cytosine base-pair
temperature of guanosine gels correlated well with ionic radius of coordinated cations [71]. It is now well established that coordination of potassium [54, 38] more rarely of sodium [43] or strontium [19] adds both thermodynamically and kinetically to the stability of G quadruplexes [72]. Potassium and strontium have similar ionic radii of approximately 1.3 Å and are believed to fit exceptionally well in the cavities between guanine tetrads. More generally, the ionic radius is a parameter that aptly describes how well guanine tetrads are stabilized by various cations. In the alkali series, the order is generally K⁺ >> Na⁺ > Rb⁺ > Cs⁺ >> Lu⁺ and for the alkali earth series, the order is Sr²⁺ >> Ba²⁺ > Ca²⁺ > Mg²⁺ [27]. Sen and Gilbert observed that addition of lithium ions exerts a strong inhibitory effect on G quadruplex formation of any kind [73].

Except for the alkali earth series, little is known about the ability of multivalent cations to stabilize G quadruplexes. It has been reported by Blume et al. that millimolar concentration of the transition cations Mn(II), Co(II), and Ni(II) counteract potassium induced G quadruplex formation [74]. This observation may explain why synthesis of poly (G) needs to be carried out in the presence of Mn(II) instead of Mg(II), which is normally used for synthesis of polynucleotides. Nonetheless, one should remember that in live cell nuclei, the free cations are dominated by four species whose approximate concentrations are as follows: K(I) (150 mM), Na(I) (5 mM), Mg(II) (0.5 mM), and Ca(II) (0.2 mM). All other cations have normal physiological concentrations in the subnanomolar range. Since most G quadruplexes greatly prefer
potassium ions it is doubtful that their formation in live cells is largely hindered by the presence of other ions. It has been reported that NH₄(I) ion can stabilize G quadruplex structure at a very low concentration namely 1 mM [3].

The perfect fit model does not stand undisputed. Hud et al. has argued that the preferred coordination of K(I) over Na(I) is driven by relative free energies of hydrogen [75]. Furthermore Nagesh et al. has observed that Tb(III) promotes G quadruplex formation cannot be attributed to the perfect fit model since its ionic radius is even larger than that of Cs⁺ [18].

1.4 G Quadruplexes and Cation Binding

Guanine rich DNA sequences and the structures that they can form are currently of great interest. The existence of these structures has been known for a long time [27]. Spectroscopic and fiber diffraction studies of poly (dG) and 5' GMP showed that guanine rich sequences form four stranded structures [76 33]. The telomeres at the ends of linear chromosomes comprise repeats of guanine rich sequences. Williamson et al. [16] proved that these guanine rich sequences form G quartets/ G quadruplex structures in vitro. Telomeres and telomerase, the enzyme that adds the guanine rich repeats to the ends of chromosomes are currently the subjects of much study as a result of the implications of telomere length regulations in cancer and cell immortality. Since G quadruplexes have been shown to inhibit telomerase [9]. G quadruplex based inhibitors of telomerase may have potential role in cancer
therapy and aging research. The focus of much of this research is on the
design and discovery of small molecules that stabilize G quadruplexes [77].
Guanine-rich repeats appear in several other locations in the human genome
including chromosome 19 [78] centromeres and fragile X syndrome repeats
[79]. Therefore quadruplex formation may also play a role in disease other
than cancer. Some *in vitro* selected inhibitors of enzymes including the
thrombin binding aptamer [40] and an inhibitor of HIV integrase [80] had
been found to form G quadruplex structures.

The first confirmed structure of a G quadruplex was reported in 1992 for
d(G₄T₄G₄) by both X-ray crystallography [54] and NMR spectroscopy [51–57].
The sequence forms a symmetric dimeric quadruplex containing four G
quartets and two T₄ loops. Subsequent NMR and crystallographic studies have
shown that guanine-rich sequences form quadruplexes with different
topologies and strand orientations depending on the sequence and the
number of strands [81, 82]. During the past year, the structure of quadruplexes
formed by two different DNA oligonucleotides has been reported. Perhaps
more importantly, the localization and effect of different monovalent cations
on the structures of these new quadruplexes [83, 84] as well as on the
structures of those by [d(G₄T₄G₄)]₂ [85, 86] and [d(G₅T₄G₃)]₂ [87] have been
extensively investigated. As cations so clearly play an important role in the
folding and stability of quadruplexes, it is of interest to experimentally
localize the ion binding sites in quadruplexes. The precise localization of
Na(Ⅰ) in the high resolution (0.96 Å) crystal structure of the tetrameric
quadruplex [d(TGGGT)]$_4$ has been reported in 1997 [48]. Solution studies of the dimeric quadruplex [d(G$_4$T$_4$G$_4$)]$_2$ [51] have shown that although the same overall fold is formed with either Na(I) or K(I) different chemical shifts are observed depending on the cation and the two conformations are in intermediate exchange on the NMR chemical shift timescale [60]. Earlier analysis of chemical shift changes in Na(I) versus K(I) for [d(G$_5$T$_4$G$_5$)]$_2$ have provided indirect evidence that one K(I) binds between each G quadruplex [75]. Studies with particle mesh ewald molecular dynamics simulations also support this conclusion [87].

In order to observe interactions between monovalent cations and DNA directly Hud et al. [86] used 15N labeled NH$_4$(I) ions as a probe for cation binding sites in the quadruplex formed by [d(G$_4$T$_4$G$_4$)]$_2$ [85 86]. NOEs observed between the NH$_4$(I) ion and guanine imino protons show that three ions bind and are located equidistant between each pair of G quartets. No NOEs were observed between any additional NH$_4$(I) ions and the loop suggesting that there are no ion binding sites in the T$_4$ loops [85 86]. The bound NH$_4$(I) ions are in slow exchange with the bulk ammonium in solution and have chemical shifts that differ significantly from that of the bulk ammonium. This is the first experimental localization of monovalent cations on nucleic acids in solution and the first direct observation of their movement into and out of the quadruplex. Hud et al. [86] and Phillips et al. [49] have noted the resemblance of guanine rich quadruplexes to a potassium channel.
Multiple repeats of the sequence GGGC appear in human chromosome 19 and also in the human adeno associated virus which specifically integrates into human chromosome 19. Thus guanine rich sequences may also play a role in viral infection and recombination. The sequence d(GGGCTTTTGGGC) is chosen as a model for these repeats. A schematic of the structure formed by the dimerization of two molecules are shown in the Figure 13. Two NMR structures of the quadruplex formed by this sequence has been solved with either Na(I) or K(I) as the monovalent cation present in solution [83, 84]. The differences between the two structures highlight the importance of the cations in the folding and stabilizing G quadruplexes.

In both structures the quadruplex is formed by the head to tail dimerization of the two hairpins with lateral T₄ loops. All strands are antiparallel to adjacent strands. In the G quartets syn and anti bases alternate around the quartet. The G quartets are similar to those observed in the other quadruplexes with antiparallel strands. It is interesting and informative to compare the structure in the presence of Na(I) with the structure of the K(I) form of this quadruplex are in slow exchange on the NMR timescale. A comparison of the downfield regions of the proton spectra show that the guanine amino and imino protons exchange more slowly in the K(I) form than in the Na(I) form as has been previously observed for [d(G₄T₄G₄)]₂ [60]. The structure of the G G G C quartets and the T₄ loops are quite different in the presence of Na(I) versus K(I). In the Na(I) form of the molecule, the G C G C quartet forms two stranded Watson Crick G C pairs and two base pairs in
Figure 13 Schematic representation of the quadruplex formed by the sequence [d(GGCT,GGGC)]$_2$. The strand topology and orientation of the bases are shown. The syn bases are shaded. The dashed line shows the base pair between guanine and cytosine that is observed in the Na(I) form but not in the K(I) form.
which the cytosine amino forms bifurcated hydrogen bonds with GN7 and GO6. The hole in the center of the G C C G tetrad is extremely small. This may affect the binding of ions to this sequence. The bases in the G C G C quartets are anti. The non Watson Crick G C pairs seen in the presence of Na(I) ions do not form in the presence of K(I) however the four bases are still anti. One consequence of the difference between the hydrogen bond patterns is a change in the stacking interactions between the G G G G and G C G C quartets. Closing the quartet with the bifurcated hydrogen bond increases the stacking interactions between the adjacent guanine and cytosine bases. This is not possible in the presence of the large K(I) ion. The structure of the T4 loops changes dramatically with the change in counter ion. The loops in the K(I) form are defined (rmsd of 0.6 – 1.3 Å for the loop residues versus 0.9 ± 1.6 Å rmsd for the loop residues in the Na(I) form) and the stacking of the thymine is different. In the Na(I) form T6 and T5 stack over C4 and C8 respectively. T6 points into solutions and T7 points towards the hold center of the G C G C quartet making a compact loop. T7 may help coordinate the Na(I) that is proposed to be bounded between the G G G G and G C G C quartets. The loops in the K(I) form are more open than in the Na(I) form. T5 and T6 stack on the adjacent cytosines and T6 and T7 stack on one another with neither pointing down towards the top of the quadruplex. Based on the calculations it has been proposed that three Na(I) binding sites between the quartets and no Na(I) binding sites in the loops and three K(I) binding sites between the quartets and two K(I) binding sites in the loops. Changing in loop
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Conformation but not topology is also observed for the Na(I) versus K(I) forms of [d(G3T4G3)]2 [87] and [d(G4T4G4)]2 [60].

Another striking example of the importance of ions for both the structure and function of multistranded DNA structures is the in vitro selected oligonucleotide inhibitors of HIV integrase [80]. The structure and efficacy of the inhibitor T30895 d(GGGTGGGGTGTTGGGG) has been studied as a function of cation type. The sequence folds to form an intramolecular quadruplex. The structure of the quadruplexes depends on the cation present. In the presence of Li(I) only the two G G G G quartets form and the G T loops are disordered. Previous studies on the effect of Li(I) on quadruplex formation have indicated that Li(I) destabilizes quadruplexes [16]. In K(I) solutions the loops fold up and stack closely with the G G G G quartets and form two G T G T quartets. There are no loops in this structure. In vitro inhibition assays show that Li(I) form a significantly less effective as an inhibitor of HIV integrase. This again illustrates the importance of cations in the folding stability and function of multistranded DNA structures.

Luminescence properties of a lanthanide ion namely Terbium(III) ion when it interacts with DNA has been extensively studied. A luminescence property of Terbium(III) ion and phenanthroline DNA triple helix systems has been reported (88). The fluorescence property of Terbium(III) ion has been utilized in this study to investigate the stability of G quadruplex DNA and its identification [18].
15 G Quadruplex DNA structures in Eukaryotic Genomes

Sequences that exhibit G quadruplex formation motifs are widely dispersed in the eukaryotic genomes but perhaps surprisingly so far there is no evidence for the occurrence of such motifs in prokaryotes. G quadruplex formation elements can be found in the telomeres [89], the immunoglobulin switch regions [56], the immunoglobulin switch regions of c-myc and other oncogenes [37, 38], the retinoblastoma susceptibility gene [36], and upstream of the insulin gene [90]. By this we cannot conclude that there are biological roles for DNA G quadruplexes built on stacked guanine tetrads, but recent discoveries of manifold human proteins that recognize them in vivo strongly suggest so. Because the structure and stability of these unusual structures of DNA are gaining importance day by day, studies have been initiated on the stability of the G quadruplex DNA when it is allowed to interact with other macromolecules and ions.

16 G quadruplex binding drugs

Structure selective targeting is critical for telomerase inhibition. The discovery and development of small molecules capable of binding to nucleic acid biotargets, typically double-stranded DNA or hybrid DNA RNA duplexes, continues to be a rewarding area for new antitumour chemotherapeutic agents. In large part, this view is reinforced by information gained during the past 30 years from studies with many current clinical anti-cancer agents (like anthracyclines, cisplatin, alkylating agents, etc) where DNA interaction or
reactivity is often implicated in their overall potency. Further, our rapidly expanding understanding of the fundamental processes involved in the malignant transformation of cells allows a wider appreciation of the key molecular events that can potentially be intercepted at the nucleic acid level using drug-based strategies.

Many classes of synthetic and naturally occurring low molecular weight agents are known that interact with DNA through a variety of distinct mechanisms including non-covalent (reversible) or covalent fixation processes. Most of the drug-based strategies have exploited the antigen approach where double-stranded DNA is targeted directly by a ligand molecule so as to interfere with template transcription or replicative processes. Binding mechanism typically involved either interaction in the minor or major grooves of the host duplex or intercalation between stacked base pairs although mixed mode binding is also often evident. Biological response is primarily governed by the effective residence time of bound molecules with cytotoxic effects arising from cellular events that require the unimpeded DNA template.

Elegant studies of the transformation of normal human cells to tumor cells have established an intimate link between tumor immortalization and activity by the enzyme telomerase [91, 92]. This enzyme serves to maintain the length of telomeres—the specialized DNA sequences at the 3' end of chromosomes that comprise tandem 5'-TTAGGG repeats in human and is found in some 85-90% of human tumors but invariably absent in somatic cells. Telomerase has
thus emerged as an obligatory anti-cancer chemotherapeutic target [93, 98] and considerable efforts are now focused on the design of agents with inhibitory activity. Documented reports that G-rich DNA sequences of this type can assemble to four-stranded tetraplex or G quadruplex structures suggested that such structures could be targeted to effect an indirect inhibition of the enzyme [99, 101]. This idea stimulated a renewal of interest in the design of DNA interactive drugs that can selectively recognize, bind and divert the telomerase substrate behavior of telomeres in whole cancer cells. Juskowiak et al. have recently shown the aggregation and binding behavior of a G quadruplex selective ligand 6a 12a diazadibenzo [a g] fluorenylium derivative by UV, Visual and fluorescence spectroscopic technique [102]. This ligand binding to G quadruplex DNA is much selective which can be used for the identification of G quadruplex DNA. Spectral characteristics have been reported to be different when the ligand aggregates with DNA at low and high NaCl concentrations. Inhibition of telomerase activity by this ligand is yet to be tested.

1.7 Biological role of DNA tetraplexes / G quadruplexes

Guanine-rich DNA sequences can assemble or fold to generate G tetraplex structures in solution at physiological concentrations of Na(I) and K(I) although their formation is yet to be established in vivo. However, despite the lack of detection in vivo, such structures are believed to play a vital cellular role. Further, the conservation of chromosomal telomeric DNA sequences may
be related to their inherent tetraplex formation [103]. This hypothesis is supported by findings that many telomeric DNA binding proteins bind to tetraplexes and or promote their formation [104-110] and may also regulate telomerase activity [111-112]. In addition, a number of helicases capable of unwinding G tetraplex DNA have recently been reported [113,114], providing further circumstantial evidence to support a cellular role for such high order DNA structure.

1.8 G quadruplex interactive ligands

All DNA tetraplexes / quadruplexes require stabilization by monovalent metal ions, where as K(I) ion is found a superior fit into the cavities of the G tetrad than Na(I) ion hence the assembly or folding of G rich DNA strands is thus favored under higher concentration of K(I) ion[115-117]. Under these conditions, telomerase processivity is inhibited as the enzyme is prevented from necessary access to its linear DNA substrate [9,118,120]. In large part, this activity has directly prompted the current quest for small molecules or ligands as telomerase inhibitors that can selectively stabilize DNA quadruplexes by mirroring the behavior of K(I) ions [93,96]. Suitable agents would find versatile applications in telomerase assay methods and as DNA directed gene probes for tetraplex formation [94].

1.9 Recent studies on G quadruplex DNA structure by NMR

Recently Phan et al have shown the two repeat human telomeric d(TAGGGTTAGGCT) sequence can form interconverting parallel and
antiparallel G quadruplex structures in solution [121] On examining the structures formed by the two repeats of Tetrahymena telomeric d(TGGGGTTGGGGT) sequence it is evident that it differs from the human sequence only by one G for A replacement in each repeat. It is shown by NMR that this sequence forms two novel G quadruplex structures in Na(I) containing solution. Both structures are asymmetric dimeric G quadruplexes involving a core of four stacked G tetrads and two edgewise loops. The adjacent strands of the G tetrad core are alternately parallel and antiparallel. All G tetrads adopt syn syn anti anti alignments which occur with 5 syn anti syn-anti 3 alternations along G tracks. In the first structure (head to head) two loops are at one end of the G tetrad core. In the second structure (head to tail) two loops are located on opposite ends of the G tetrad core. In contrast to the human telomere counterpart the proportions of the two forms here are similar for a wide range of temperatures their unfolding rates are also similar with an activation enthalpy of 153 kJ/mol.

Recently Phan et al. demonstrated by NMR that the two repeat human telomeric sequence d(TAGGGTTAGGGT) can form both parallel and antiparallel G quadruplex structures in K(I) containing solution [122]. Both structures are dimeric G quadruplexes involving three stacked G tetrads. The sequence d(TAGGGUTAGGGT) containing a single thymine to uracil substitution at position 6 formed a predominantly parallel dimERIC G quadruplex with double chain reversal loops. The structure is symmetric and all guanines are anti parallel. Another modified sequence
d(UAGGGT(Br)UAGGGT) formed a predominantly antiparallel dimeric G quadruplex with edgewise loops. The structure is asymmetric with six syn guanines and six anti guanines. The two structures can coexist and interconvert in solution. For the latter sequence, the antiparallel form is more favorable at low temperatures (<50°C) while the parallel form is more favorable at higher temperatures. At temperatures lower than 40°C the antiparallel G quadruplex folds faster but unfolds slower than the parallel G quadruplex.

1.10 G quadruplex structure and its relevance to cancer research

Recently, much work has been carried out showing a relationship between G quadruplex structure and cancer. Most of the work has concentrated on the aspects and factors which stabilize the G quadruplex structure and thereby inhibiting the telomerase activity in vivo. The work by Ishukawa et al. [123] emphasizes that telomere interactive compounds such as TMPyP₄ are expected to inhibit telomerase and thus to be a potential anti-cancer drug. One of the porphyrins, pPyTTP, is found to bind to the G quadruplex DNA and to increase the Tₘ of the G quadruplex. The positive sign of induced CD for the complex suggested pPyTTP bound to and stabilized the G quadruplex DNA possibly by groove binding, distinct from that of TMPyP₄.

Work by Riou et al. [124] implies that peculiar sequence of telomeric DNA composed of repetitions of the GGTTAG motif allows the formation of an
unusual DNA conformation based on guanine quadruplex (G quadruplex) Small molecules that bind and stabilize telomeric DNA under its G quadruplex conformation are able to impair telomerase activity Several recent reports have shown that G quadruplex ligands could block telomerase activity in cancer cells and represent a new experimental approach to limit cancer growth The intracellular existence of G quadruplex structure is still controversial since no direct proof allowed to establish its reality Many sequences of nucleic acids in the mammalian genome are able to form a G quadruplex in vitro and several proteins have been described to interact in vitro with G quadruplex These data indicated that G quadruplexes are members of a family of target structures larger than that initially described at telomeres and raised the question of the selectivity and therapeutic index of their ligands in the context of an antitumor therapy

A selective interaction of a very well known DNA binding drug namely Ethidium with G quadruplex DNA has been studied by Rosu et al. [125] in detail According to them the telomeric G rich single stranded DNA can adopt in vitro an intramolecular quadruplex structure which has been shown to directly inhibit telomerase activity The reactivation of this enzyme in immortalized and most cancer cells suggests that telomerase is a relevant target in oncology and telomerase inhibitors have been proposed as new potential anticancer agents It has been recently found that the selectivity of four ethidium derivatives and ethidium itself toward different G quadruplex species with electrospray mass spectrometry and competitive equilibrium
dialysis and evaluated their inhibitory properties against telomerase. A selectivity profile may be obtained through electrospray ionization mass spectrometry (ESI MS) which is in fair agreement with competitive equilibrium dialysis data. It also provides unambiguous data on the number of binding sites per nucleic acid (maximal number of two ethidium derivatives per quadruplex in agreement with external stacking).

A detailed study on the efficacy of telomerase inhibitor (TMPyP₄ [tetra (N methyl 4 pyridyl) porphyrin chloride] a G-quadruplex intercalating porphyrin as a potential therapeutic agent for multiple myeloma is carried out by Shammas et al [126]. Further few studies by Zhang et al [127] showed that DODC alone could induce hairpin G quadruplex formation and inhibit telomerase activity in a dose dependent manner. Compared with HT1080 cells, 293 cells are more sensitive to cell growth inhibition apoptosis and were less sensitivity to telomerase activity. These results indicate that DODC can synergistically enhance the apoptosis induced by arsenic suggesting the increased cell senescence in response to arsenic is induced by an altered telomere state rather than by a loss of telomerase. Thus clinical application of combination treatment with arsenic and telomerase inhibitor may have potential role in cancer therapy. Gomez et al [128] provide evidence that telomerase activity and telomere length are key cellular determinants for resistance to G-quadruplex ligands.

Mokbel et al [129] have recently shown the role of telomerase inhibitors in treatment of cancer. Telomerase is a ribonucleoprotein that maintains
telomeres and is essential for cellular immortality and tumor growth. The differential expression of telomerase in cancer cells makes it an attractive therapeutic target. Anti-sense oligonucleotides directed against the RNA template of hTR and small molecules that can interact and stabilize the G quadruplex representing promising therapeutic strategies. Human trials investigating the potential role of the catalytic subunit hTERT as a universal cancer vaccine have already commenced.

A G quadruplex binding ligand 3,3 diethyloxadicarbocyanine iodide induces mitochondrial mediated apoptosis but it does not decrease telomerase activity in nasopharyngeal carcinoma NPC TW01 cells. has been shown by Lu et al. [130] Grand et al. [131] have demonstrated that a parallel G quadruplex structure in the c-myc promoter functions as a transcriptional repressor element. Furthermore, a specific G to A mutation in this element results in destabilization of the G quadruplex repressor element and an increase in basal transcriptional activity. They have also found that approximately 30% of tumors contain one of two specific G to A mutations but not present in the surrounding normal tissue. The mutation will lead to destabilize the parallel G quadruplex which would be expected to give rise to abnormally high expression of c-myc in these cells.

A G quadruplex interactive agent telomestatin (SOT 093) which induces telomere shortening with apoptosis and enhances chemo sensitivity in acute myeloid leukemia was studied by Sumi et al. [132] Telomerase the ribonucleoprotein enzyme maintaining the telomeres of eukaryotic
chromosomes is up regulated in the vast majority of human neoplasias but not in normal somatic tissues. Therefore, the telomerase complex represents a promising universal therapeutic target in cancer. Telomeric G-rich single stranded DNA can adopt \textit{in vitro} an intramolecular quadruplex structure which has been shown to inhibit telomerase activity. Sumi \textit{et al.} examined G quadruplex interactive agent telomestatin (SOT 095) for its ability to inhibit the proliferation of human leukemia cells including freshly obtained leukemia cells. Telomere length is determined by either the terminal restriction fragment method or flow FISH and apoptosis is assessed by flow cytometry.

Interaction of cryptolepine and neocryptolepine with different unusual DNA structures has been studied by Mergny \textit{et al.} [133] The study emphasizes that cryptolepine and neocryptolepine both a GC base specific intercalators has weak telomerase inhibition and exhibit a significant preference for triplexes over quadruplexes.

Many analytical techniques were used to study the interaction of G quadruplex DNA with other molecules. But among them a recently well established and very sensitive mass spectroscopic technique namely Electrospray Ionization Mass Spectrometry (ESI MS) is very frequently used in studying the DNA interaction with other molecules. Gross \textit{et al.} [134] have recently studied the G quadruplex DNA interaction with metal ions using ESI MS technique.
Thus telomestatin provides the novel therapeutic molecular target for cancer chemotherapy. Telomestatin induced telomere shortening but its ratio is extremely faster than that observed in physiological telomere shortening. Shinya [135] work on telomestatin reveals that it is one of the potential candidate for cancer therapy in future. The different biological effects of telomestatin and TMPyP₄ can be attributed to their selectivity for interaction with intramolecular or intermolecular G quadruplex structures. This has been studied in detailed by Kim et al., [136]

Telomerase is a ribonucleoprotein enzyme that maintains protective structures at the ends of eukaryotic chromosomes. Nakajima et al. [137] examined the impact of telomerase inhibition by the dominant negative human catalytic subunit of telomerase (DN hTERT) on the biological features of acute leukemia.

1.11 I motif quadruplexes

It was proposed in the 1960s that polycytidylic acid would form parallel duplexes at low pH. [138] Cytosine rich sequences are interesting because they are the complements of the guanine rich telomere sequences and because cytosine rich sequences are found in centromeres. The first detailed structure of cytosine rich sequence was a big surprise [139]. The oligonucleotide d(TCCCCC) forms a tetrameric four stranded structure in which one parallel C⁺C duplex with hemiprotonated C⁺C pairs intercalates into a second duplex which is oriented antiparallel to the first duplex.
motif) [140] The base planes of the stacked $C^+C$ pairs are perpendicular to each other. The Figure 14 shows a schematic of the stacked $C^+C$ pairs. The existence of the I motif demonstrates most dramatically the wide range of structures that DNA can form. Two solution structures and one X-ray structure of sequences that form the I motif were solved during the past year, which is shown in Figure 15. The three I motif structures are formed by four, two or one strands. The I motif core is similar in all three structures. The most interesting differences are in the end effects observed in the loops of the folded structures and at the ends of the quadruplexes in the tetrameric structures.

The crystal structure of the quadruplex formed by the oligonucleotide d(AACCCC) was solved in Rich's laboratory [141]. The cytosines in four stands come together to form a tetrameric I motif core. There are two different I motif quadruplexes in the asymmetric units of the crystals; one in which the terminal $C^+C$ pair is the 3 pair and the other in which the terminal pair is 5 pair. This is the first example of a 3 terminal $C^+C$ pair. There are four grooves: two broad and flat major grooves and the two extremely narrow minor grooves. The helical twist between covalently linked $C^+C$ pairs is 12.16. This I motif has the narrowest minor grooves of any structure solved to date. The wide grooves are also broader and flatter than those observed in the solutions structure.

The striking feature of this structure is the arrangement of the adenine and the resultant stacking of the quadruplexes. The terminal adenines form two
Figure 14  The pairing and stacking of the C\textsuperscript+\ C base pairs in the I motif
Figure 15  The folding topology and base pairing of the four I motif quadruplexes
different clusters. The planes of AA base pairs in the cluster were perpendicular to the planes of the $C^+C$ pairs in the central core. Noncovalently attached $C^+C$ cores stack upon neighboring clusters. The type 1 (or 5 terminal) quadruplexes stack along the $z$ axis on the top of the adenine clusters formed by the adenines of the type 2 (or 3 terminal) $C^+C$ cores which stack along the $x$ axis. The adenine clusters contain both parallel and antiparallel base pairs and there are six different base pairs. Some of the AA interactions observed are similar to the adenine platforms seen in the crystal structure of the P4 P6 group I ribozyme [142].

Cytosine rich sequences also appear in the centromere of some human chromosomes. Reid's laboratory [143], which has a long standing interest in centromeric sequences recently solved the solutions structure of the oligonucleotide $d(TCCGTTCGA)$. This sequence is part of the region of the centromere called the CENP B box, the binding site of the centromere protein B. This oligonucleotide in the absence of the complementary strand will fold back on itself and form a dimeric I motif quadruplex. The hairpins pair in a head to head fashion with four $C^+C$ pairs formed between parallel strands of adjacent hairpins. The geometry of the base pairs, the orientation of the strands and the helical parameters in the $C^+C$ core are similar to those in other I motif structures. Again the most striking feature of this structure is not the I motif core, but rather is the structure of the GTTT loops. The two $G_8$ residues pair and are approximately parallel to the adjacent $C_9C_8$ pair. $T_8$ from hairpin 1 form a G T wobble pair with $G_8$ from hairpin 2 which is also
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Paired with \( G_5 \) from strand 1 creating a novel T G G T tetrad. \( T_6 \) faces out into solutions. \( C_2 \) and the 5' end of the \( C^+ C \) core is weakly paired. \( T_1 \) \( C_2 \) and \( A_{11} \) form a series of cross strand stacking interactions, but none of the bases appear to be hydrogen bonded. Despite the lack of evidence for base pairing, the stacking interactions are such that the bases continue the perpendicular arrangement that is characteristic of the I motif.

Han et al. [144] solved the solution structure of the oligonucleotide \( \text{d}(5\text{mCCT_3CCT_3ACCT_3CC}) \) which folds back on itself to form a monomeric I motif with four stacked \( C^+ C \) pairs. The T3A loops crosses one of the wide grooves \( T_3 \) and \( A_{11} \) form a propeller twisted reversed Hoogsteen base pair in which \( A_{11} \) stacks on the adjacent \( C_7^+ C_{18} \) pair. \( T_3 \) and \( T_{18} \) form a T T wobbles that stacks under the \( C_2^+ C_{13} \) pair and continues the perpendicular stacking pattern of the I motif core. The two base loops are sufficient to span the narrow groove of the I motif core. The base pair life times and imino proton exchange rates of the \( C^+ C \) pairs are similar to those observed for the tetrameric I motif [139], indicating that single strand folds into an I motif does not affect the stability of the \( C^+ C \) pairs.

The imino proton exchange rates for the paired thymine are consistent with the formation of the A T and T T pairs. These three different I motif structures show how the choice of linker can affect the resultant structure and how crystal packing interactions can cause long range order in the structures of relatively small DNA fragments. It is interesting to note that in both the
dimeric and monomeric quadruplexes the base pairs that form at the loops are parallel to the adjacent C^+ C pairs.

More than 85% of c-myc transcription is controlled by the nuclease hypersensitive element III (1) upstream of the P1 promoter of this oncogene. The purine rich sequence in the anti sense strand forms a G quadruplex which has been recently implicated in colorectal cancer and is proposed as a silencer element [131]. Similarly a C rich DNA strand form C quadruplex whose thermodynamics and proton/counterion effect are also studied. Mathur et al [145] reported the thermodynamic parameters for folding of the pyrimidine rich DNA fragment from this region into a C tetraplex. At 20° C Mathur et al has observed a delta G of 10.36 +/- 0.13 kcal mol\(^{-1}\) with favorable enthalpy (delta H = 75.99 +/- 0.99 kcal mol\(^{-1}\)) and unfavorable entropy (T delta S = 65.63 +/- 0.88 kcal mol\(^{-1}\)) at pH 5.3 in 20 mM NaCl for tetraplex folding. Similar characteristic stabilizing enthalpy and destabilizing entropy were observed at other pH and ionic strengths. Folding was induced by uptake of about two to three protons per mole of tetraplex while a marginal (0.51 mol/mol) counter ion uptake was observed. In the context of current understanding of c-myc transcription it is envisage a role of the 1 motif in remodeling the G quadruplex silencer.

1.12 Relevance of the present study

Initially work on G quadruplex DNA has started to find out the unusual behaviour of guanosine residues in solution. Many researchers have worked
on finding out the structure and favorable conditions for the formation of G quadruplex complex. While the studies are progressing in this direction, guanosine stretches have been observed and reported in many vital regions of DNA. There is a renewed interest in G quadruplex structures due to their putative biological regulatory functions. These G quadruplex structures have been shown to directly inhibit telomerase elongation in vitro. Therefore, a drug or metal ion that stabilizes quadruplexes could interfere with telomere elongation and replication of cancer cells. The enzyme, namely telomerase, is expressed in tumor cells but not in most somatic cells. Keeping this as a main objective, primary importance is given to the structure and stability of G quadruplex DNA formed by chemically synthesized oligonucleotides when they are allowed to interact with different drugs and metal ions. In this part of the work, attempts were made to find a suitable ion which can stabilize G quadruplex structure more efficiently at low concentration. It has been identified that NH₄(I) ion is more efficient and capable of stabilizing the G quadruplex structure at low ion concentration when compared with other competing ions. It is also found that Terbium (III) ion could not stabilize the G quadruplex structure alone. It requires an alkali metal ion to stabilize the structure. Studies were performed with 2 different DNA stretches observed in *Tetrahymena*. Drug interaction with G quadruplex DNA has important role in finding and designing a suitable drug for cancer therapy. G quadruplex DNA interaction with drugs like acridine, *bis* acridine, actinomycin D, Hoechst 33258, has revealed many new aspects. Hence, the finding of this work is useful.
for designing drugs to cancer. All the studies are carried out using CD fluorescence gel shift assays and other modern analytical techniques. ESI MS gel electrophoretic studies were performed to find out the nature and purity of the oligonucleotides.

1.13 Objectives of the present study

The main objectives for starting and carrying out this part of the work are given here under. The main objectives of this part of the work are finding the conditions and factors responsible for the unusual stability of $\text{NH}_4(\text{I})$ G quadruplex DNA and to observe the effect and interaction of different drugs ions on G quadruplex DNA. Folding the telomere into a G quadruplex structure could inhibit cancer because the G quadruplex structure must first be unfolded in order to render the telomere end accessible to the telomerase enzyme. It is therefore thought that if telomeres could be stabilized using such a structure, the cells could be prevented from the infinite proliferation characteristic of cancer. A telomere end folded into a G quadruplex structure can be stabilized by stacking certain small molecules onto the ends of the G quadruplexes. Conclusive evidence for the formation of these unique G quadruplex structures within human cells has been provided and has led to a rational search for small molecules that can selectively interact with G quadruplexes and stabilize the structure.

Different classes of molecules have been shown to inhibit the unraveling of G quadruplex structures through their interaction with the G quadruplex...
DNA On the basis of observations that potassium ions can stabilize these structures small molecules that mimic their effect have been designed and found to inhibit telomerase activity. Attempts are made to stabilize G quadruplex at lower concentrations using ammonium ions. After the discovery of G quadruplex interactive telomerase inhibitors based on anthraquinone a number of other compounds have been identified. These include the fluorenones bi substituted acridines and cationic porphyrins etc. In this study NH₄(I) G quadruplex DNA is allowed to interact with different drugs like acridine bis acridinehoechst 33258 and actinomycin D and its stability is monitored. The main objectives for undertaking this work are briefed hereunder. They are

- To observe the stability of G quadruplex DNA in presence of a bigger ion namely terbium(III) ion
- Study the effect of an extra T residue at the bend of G quadruplex structure
- To observe the stability of G quadruplex structure in presence of low concentration of ammonium ion
- To find the factors which effects the formation of inter and intramolecular G quadruplex structures
- Effect of different drugs on the inter and intramolecular G quadruplex structure
- Effect of thermal treatment on the formation of higher order structures
Find the effect of size and charge of the interacting drug on the stability of G quadruplex structure

Study the effect of charge and ionic radii of central stabilizing ion on the stability of G quadruplex structure