Determinants of DNA Quadruplex Structural Type: Sequence and Potassium Binding†

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ABSTRACT: There are DNA sequences which adopt the same quadruplex structural type in the presence of sodium as in the presence of sodium and potassium. There are also sequences that appear to have a requirement for the presence of potassium for the adoption of a particular quadruplex structural type. Information about the basis for these potassium effects has been obtained by examining the structures of a set of DNAs with differing numbers of loop residues and different lengths of runs of dG residues in the presence of sodium alone and in the presence of potassium and sodium. On the basis of the results, obtained primarily via solution-state NMR, it appears that very small loops favor parallel stranded quartet structures which do not require the presence of potassium. DNAs with loops of two to four residues and runs of two dG residues can form quadruplex structures of the "edge" or "chair" type in the presence of potassium but not in the presence of sodium alone. When all of the loops contain four residues, a "crossover" or "basket" type structure can be formed in the presence of sodium as well as in the presence of sodium and potassium. Structures with runs of three or four dG residues and with loops from two to four residues can form basket or crossover type structures in the absence of potassium. The presence of a purine in a loop can block both potassium binding and formation of chair type structures. Modeling of the interactions of cations with these quadruplex structures indicates that the potassium ions required for chair type structures interact with a terminal quartet and residues in the adjacent loop.

Many nucleic acids require one or more specific ions to adopt their active structures (*1*−*3*). For example, magnesium ions appear to be essential for the activity of some ribozymes (*4*). Magnesium also plays a central role in stabilizing the structure of intron sequences (*5*) as well as the structures, and hence functions, of transfer RNAs (*6*). Potassium has a role in some DNA quadruplex structures (*7*−*10*). For example, d(GGTTGGTGTGGTTGG) adopts an intramolecular chair type quadruplex structure (*11, 12*), which is essential for its ability to inhibit thrombin, only in the presence of potassium (*13*).

There is a growing interest in DNA quadruplexes as they are important as lead molecules in drug design (*14*−*16*) and as a structural motif potentially adopted by telomere (*17, 18*), fragile X (*19*−*22*) immunoglobin switch regions, centromere DNA, and other biological systems (*23*−*29*). Quadruplex DNAs are also being examined as possible drug targets for telomerase inhibition and other roles (*30*−*33*). The unusual structural and electrostatic properties of DNA quadruplexes may be prime determinants of their biological activities.

The structures and stabilities of quadruplex DNAs are known to be dependent on the nature and concentrations of monovalent cations present in the solution (*10, 18, 34*−*40*). These and other studies (*41*−*45*) have indicated that monovalent cations have specific effects on the properties of quadruplex DNA that are quite different from those observed for duplex DNA, tRNA, or pseudoknot RNA. Divalent and trivalent ions also interact with DNA quadruplex structures (*38, 46, 47*), and we have recently shown that manganese preferentially binds to the narrow grooves of two distinct types of quadruplex DNA (*48, 49*).

Structural information on many DNA quadruplexes is now available including those formed by four parallel strands (*50*−*54*). Quadruplexes can also be formed by dimers with the loops crossing over in a basket type structure or as an edge dimer as illustrated in Figure 1 (*55, 56*). Crossover and edge type structures can also be formed by intramolecular folding as depicted in Figure 1 (*11*−*13, 57*−*61*). The dG residues forming a quartet can be all *anti* as is the case for the parallel stranded structures. The alternation *syn*−*anti*−*syn*−*anti* is associated with chair type structures, and the alternation *syn*−*syn*−*anti*−*anti* is associated with crossover type structures as indicated in Figure 1. In addition, both *syn*−*syn* and *syn*−*anti* alternations from 5′ to 3′ have been observed in crossover type structures.

The results to date on the effects of potassium suggested to us that there are two classes or types of potassium−DNA quartet interactions. The quartets in a parallel stranded structure essentially make a square with four medium width grooves (*52*). The parallel strand structural type can be formed in the presence of sodium and in the presence of potassium. The quadruplex structures of the crossover type

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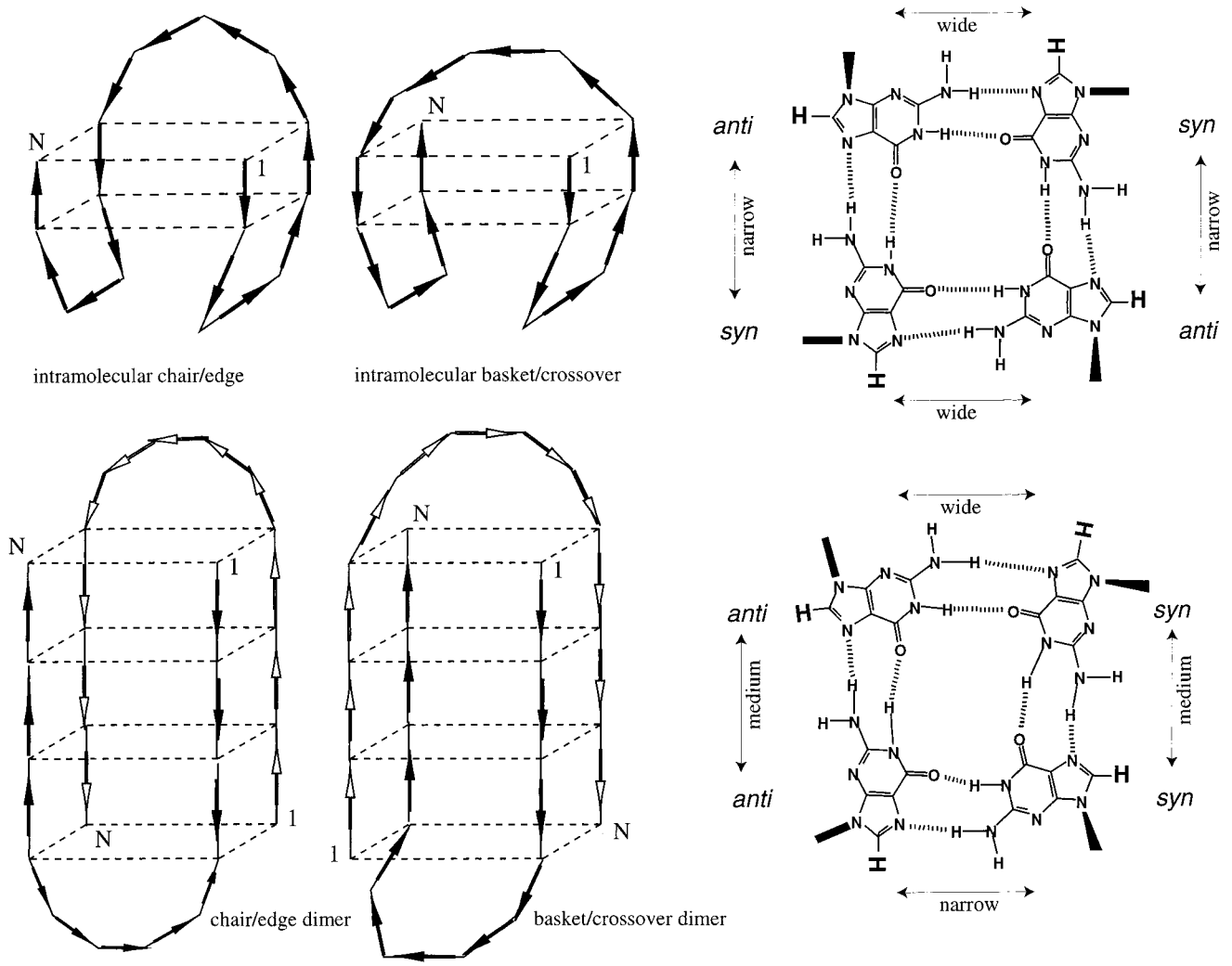

FIGURE 1: Intramolecular and dimer forms of chair/edge type quadruplexes and intramolecular and dimer forms of crossover/basket type quadruplex structures. Also shown are the arrangements of the dG residues within the quartets of the two types of quadruplex structure. The chair/edge type structures have *anti*−*syn*−*anti*−*syn* alternation of the dG within each quartet and two narrow and two wide grooves as shown. The crossover/basket type structures have *anti*−*anti*−*syn*−*syn* alternation within each quartet and have two medium, one wide, and one narrow groove as shown. A parallel strand structure has four medium width grooves.

can be formed in the presence of sodium alone as well as in the presence of sodium and potassium. The quartets in this type of structure form a parallelogram arrangement as shown in Figure 1. Quadruplex structures of the chair type have, to date, only been observed in the presence of potassium. The quartets in this type of structure form a rectangular arrangement as depicted in Figure 1. All DNA quadruplex structures, with dG quartets, determined so far follow this pattern suggesting that a *requirement* for potassium may be limited to chair type structures. To test whether this correlation of structural form and potassium requirement can be generalized, the structural features of a series of DNAs have been examined in the presence of sodium alone and in the presence of sodium and potassium.

Quadruplexes which contain dC residues in the quartets adopt distinct structures and have different cation dependences than those formed with dG residues in the quartets (*62*, *63*). The DNA d(GGGCTTTTGGGC) can form a head-to-tail quadruplex structure that is an edge dimer that contains both GGGG and GCGC quartets. This quadruplex structure can be formed in the presence of sodium alone, and the quadruplex structural type is not changed by the presence of potassium.

DNAs that form quadruplexes, with dG quartets, can be divided into the dG_n regions that form the quartets and the intervening regions that form the loops. The role of loop length on the potassium dependence has been examined via a series of DNAs with two, three, or four residues in the loop regions. These sequences are arranged into two-dimensional representations of intramolecular and dimer quadruplex structures in Figure 2. DNAs with the potential to form different numbers of quartets, but all with the same loops, have also been studied to examine the roles of the number of quartets on the potassium dependence and the sequence examined here are shown in Figure 2. The dependence of the potassium effect on the sequences of the loops has also been examined.

The results on these sequences will allow testing the hypothesis that potassium binds only to, or between, the quartets. This hypothesis also predicts that variations in the loops will have a modest effect on potassium binding. An alternative hypothesis of potassium binding to loop residues and the adjacent quartet would predict significant differences in potassium effects due to variations in the length and sequence of the loop regions. This hypothesis on the importance of the loop residues would also be consistent with

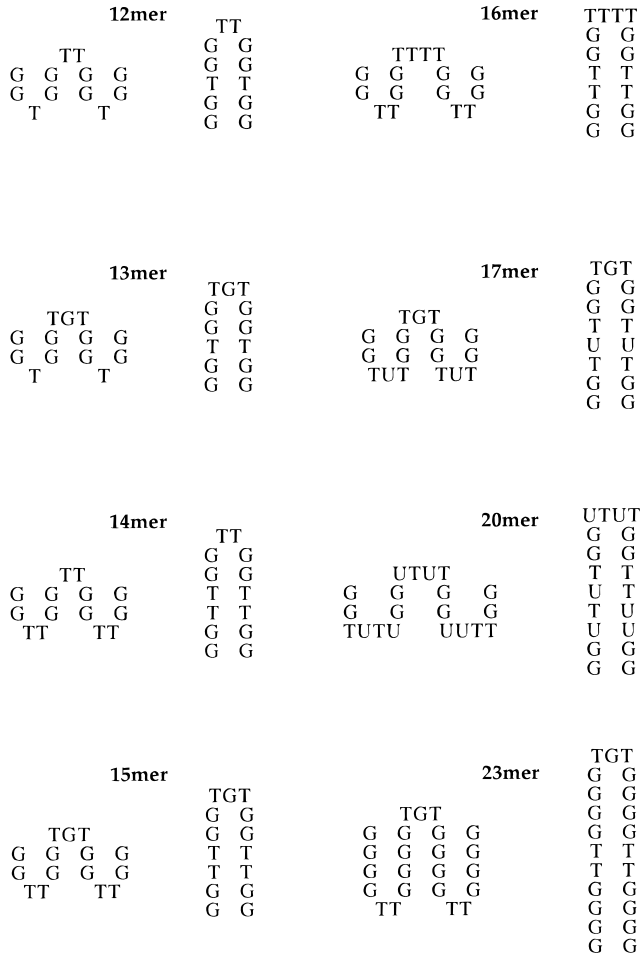

FIGURE 2: Sequences of the eight DNAs examined here. The "W" arrangement is the one that the DNA might adopt if it forms an intramolecular quadruplex structure of the types shown in Figure 1. The inverted "U" arrangement is the one that the DNA might adopt if it forms a dimer quadruplex structure of the types shown in Figure 1.

differences in potassium binding effects as the number of quartets is increased due to a tradeoff between potassium binding to the loop and adjacent quartet and the preferred structural preference of the larger number of quartets.

The binding of main interest here is the type that is required for a specific type of quadruplex structure to form. There is another class of binding which is evidenced by modest structural changes between the potassium and sodium forms of DNA quadruplexes (*34*, *62*) in which the addition of potassium induces structural changes within a type of quadruplex structure.

MATERIALS AND METHODS

DNA Purification and Sample Preparation. The DNA samples d(GGGGTTTTGGGG), referred to as 12mer, d(GGTGGTGTGGTGG), referred to as 13mer, d(GGTTGGTTGGTTGG), referred to as 14mer, d(GGTTGGTTTTGGTTGG), referred to as 16mer, d(GGTUTUGGUTUTGGUUTTGG), referred to as 20mer, d(GGGGTTGGGGTGTGGGGTTGGGG), referred to as 23mer, and d(GGTUTGGTGTGGUTTGG), referred to as 17mer, were obtained from Integrated DNA Technologies, Inc., Coralville, IA. The DNA d(GGTTGGTGTGGTTGG), referred to as 15mer, was obtained from Gilead Sciences, Foster City, CA.

All the DNAs were purified by HPLC and then lyophilized. The samples were dissolved in 200 mL of 140 mM NaCl and 20 mM perdeuterated Tris at pH 7.0 and then precipitated by adding 1.8 mL of cold dehydrated 200 proof ethanol to the sample in a 2-mL vial. This solution was then cooled to −4 °C for approximately 1 h. The precipitated DNA was centrifuged to produce a white pellet. This precipitation procedure was repeated three times to produce a DNA sample free of potassium. NMR and HPLC on these samples detected no impurities.

The NMR samples consisted of 100 A_{260} of DNA in 500 μL of 140 mM NaCl and 20 mM perdeuterated Tris buffer at pH 7.0 in a grade-6 NMR tube (Scientific Glassware, Vineland, NJ). An A_{260} is an absorption of 1 at 260 nm with the sample in a 1-cm path length cell. Each sample was then dried in the NMR tube using N_2 gas and then redissolved with 500 mL of ^2H_2O. The pH was then checked and adjusted, if necessary, to 7.0.

The samples were annealed before the first addition of potassium and after each addition of potassium. The slow anneal process involved placing the sample in a 80 °C water bath followed by allowing the entire water bath to slowly cool to room temperature. The fast anneal process involved placing the sample in a 80 °C water bath followed by transfer to a 5 °C water bath. For the 16mer and 17mer the short anneal process produced a single DNA conformation, while the slower cool procedure produced mixtures. For all of the other DNAs both rapid and slow cooling gave the same results.

The titrations were conducted by adding KCl in ^2H_2O to each DNA sample. The KCl concentration was determined by atomic absorption to be 9.9797 ± 0.0004 mM by Galbraith Laboratories, Inc., Knoxville, TN. The concentration based on weight was 10.0000 mM.

NMR Experiments. All of the one-dimensional NMR experiments were carried out on a Varian 400-MHz Unity Plus spectrometer. A standard one-dimensional experiment was used with the samples at 15 °C; 2048 transients were used with a spectral width of 8000 and a delay time of 1 s.

ROESY,[1] TOCSY, and NOESY experiments were conducted using a Varian Inova 500-MHz spectrometer using States-Haberkorn data acquisition. The number of increments given below is for each of the two data sets that make up the complex data. ROESY spectra were acquired at 15 °C using a delay time of 1.0 s, a mixing time of 55 ms, and a spectral width of 5000 in both dimensions, and 128 transients were acquired for each of the 250 increments of t_1. TOCSY spectra were acquired in ^2H_2O at 15 and 35 °C with a delay of 1.0 s and a mixing time of 60 ms. The spectral width was 6000 Hz in both the F_1 and F_2 dimensions, and 48 transients were acquired for each of the 400 increments of t_1. NOESY experiments were conducted at 15 °C with a mixing time of 250 ms and a delay time of 1 s. A spectral width of 5000 Hz was used in both dimensions with 128 transients acquired for 300 increments of t_1. Gaussian weighting functions in both dimensions were used.

Refinement of the Energies and Positions of the Potassium Binding Sites. X-PLOR 3.1 (*64*) was used to determine the

[1] Abbreviations: NOESY, nuclear Overhauser effect spectroscopy; NOE, nuclear Overhauser effect; TOCSY, total correlation spectroscopy; ROESY, rotating frame Overhauser effect spectroscopy.

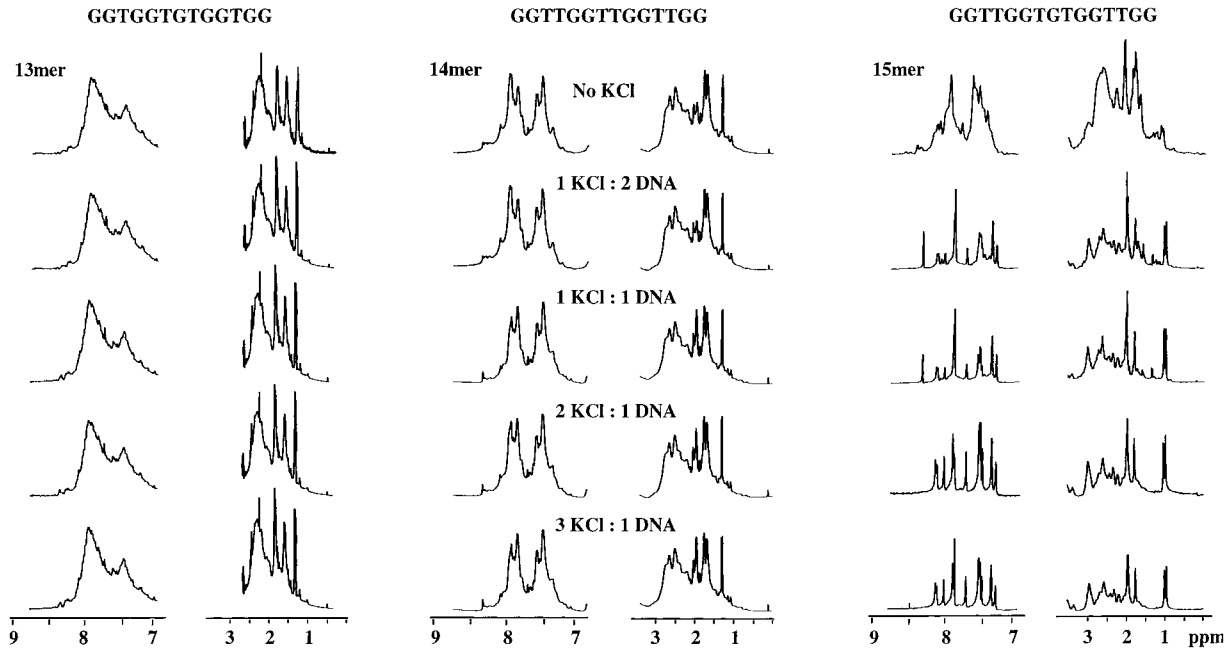

FIGURE 3: One-dimensional spectra obtained during the titrations of the 13mer, 14mer, and 15mer samples. The ratio of DNA to potassium is indicated for each step of the titration.

minimum energy positions of the potassium ions using the force-field CHARMM. A van der Waals radius of 1.96 Å and a charge of +1 were used for each potassium ion. The standard distance-dependent dielectric function was also included in the nonbonded terms with an ϵ_0 of 64. The switching function for the nonbonded interactions was operative between 7.5 and 10.0 Å. The nonbonded interaction cutoff minimum was 0.5 Å, and the maximum was 10.0 Å with a smooth transition. The DNA−potassium complexes were subjected to a 500-step minimization allowing only the potassium to move while the DNA was held rigid.

Energy minimizations were run using various starting positions for the potassium ions, and the final positions and energies were found to be independent of the initial coordinates of the potassium. Ten starting positions for each potassium from 5 to 10 Å away from the surface of the DNA were used. Five of these were on the G_{15} side and five on the G_6 side of the DNA. The minimizations were carried out first with one potassium ion per quadruplex and then with two per quadruplex. The position of the first binding site did not change, upon addition of the second potassium, for either DNA. Additional minimization did not change the positions of the potassium ions.

Energy Minimization of Adenine-Containing DNAs. The thymines in positions 3, 4, 7, and 9 were individually replaced with adenine using Biopolymer, part of Insight II 97.0. The adenines were positioned to have the same orientation in space as the thymines they replaced. Otherwise the structure of the DNA was not changed and contained two potassium ions located in the positions described above. A 500-step rigid body minimization, using X-PLOR 3.1 with the CHARMM force field, was used to minimize the energy of the complex by allowing only the position of the adenine residue to vary and using the following parameters. The distance cutoff for the hydrogen-bonding interactions was set to 4.5 Å, and the switching function was operative from 4.50 to 7.50 Å. The nonbonded interaction cutoff was set to 10.0 Å maximum and 0.5 Å minimum. The switching function for the nonbonded interactions was operative over the range from 7.50 to 10.0 Å. The standard distance-dependent dielectric function was also included in the nonbonded terms with an ϵ_0 of 64. The total energy, in kcal/mol, of the minimized structures for each DNA with adenine, E_A, or with thymine, E_T, at position 3 is $E_A = 2.103 \times 10^5$, $E_T = 215.347$. At position 4 the energies are $E_A = 4.601 \times 10^5$, $E_T = 196.747$; at position 7 they are $E_A = 1.521 \times 10^5$, $E_T = 211.590$; and at position 9 they are $E_A = 1.370 \times 10^5$, $E_T = 188.630$.

RESULTS AND DISCUSSION

Each DNA sample, originally in 0.1 M NaCl, was titrated to determine whether a structural change is induced by the presence of potassium as evidenced by significant changes in the chemical shifts and line widths of the nonexchangeable protons. The results of the titrations will also allow determination of the number of potassium ions needed to induce the structural changes and whether the changes occur sequentially or cooperatively. Preliminary experiments with the 15mer indicated that the addition of potassium led to variable results when carried out at room temperature. Annealing the sample after each potassium addition led to reproducible results, and all of the samples were annealed before each measurement.

Potassium Is Needed for the Structure of the 15mer. Previous structural studies on the 15mer, on its interactions with thrombin, and on its interactions with manganese (*11*−*13, 48, 49*) have shown that in the presence of potassium this molecule adopts an intramolecular chair type structure as shown in Figure 1. This structure is kinetically stable as indicated by the imino protons having exchange times with water which are orders of magnitude slower than those observed for duplex DNA (*11, 12*). The sample, in 0.1 M NaCl alone, has a largely unresolved one-dimensional spectrum of the nonexchangeable protons in all regions as shown in Figure 3. This sample may be forming a set of

parallel stranded structures that have different staggered arrays of monomers. This would allow a diverse range of parallel stranded structures to be present giving rise to unresolved spectra.

The presence of potassium induces striking changes in the chemical shifts as shown in Figure 3. The sample in the presence of 0.5 potassium per DNA has a spectrum that is a mixture of sharp and broad resonances. A spectrum indicative of a single well-ordered structure is obtained with one potassium per DNA. The spectrum obtained with one potassium per DNA contains signals that are not observed from either the potassium-free or the potassium-saturated sample. For example, there is a resonance, at 8.4 ppm, that is observed from neither the potassium-free nor potassium-saturated samples but is observed when there is one potassium per DNA. This indicates that there is an intermediate with distinct structural features. The spectrum obtained in the presence of two potassium ions per DNA is well-resolved and contains one sharp resonance from each nonexchangeable proton in all regions of the spectrum as previously demonstrated (*11*, *12*). The addition of more than two potassiums per DNA does not induce any appreciable additional change in the spectrum. Levels of more than five potassiums per DNA were not examined here.

The results on the 15mer can be summarized as follows. Potassium induces a significant change in the structure of this DNA as indicated by large changes in chemical shifts and line widths. Two potassiums per DNA are needed to induce the entire structural change, and the two potassiums bind sequentially. The structure in the presence of one potassium per DNA contains unique structural features found in neither the potassium-free nor potassium-saturated DNA. Thus, there is a difference in binding strength between the two sites, and binding at both sites is needed for the complete structural change.

DNAs without a Potassium Requirement. The titration of the 14mer, shown in Figure 3, shows that the chemical shifts of this DNA do not change appreciably upon the addition of potassium to the sample in 0.1 M NaCl. There is a general resemblance between the spectra of the 14mer and 15mer samples in the absence of potassium. The results on the 13mer shown in Figure 3 are much the same as those for the 14mer in both the presence and absence of potassium. It appears that both the 13mer and 14mer form a set of parallel stranded quadruplexes that have different staggered arrays of monomers.

The potassium titration of the 20mer, shown in Figure 4, shows that this DNA forms a well-defined structure in the presence of sodium alone and that there is no appreciable change in the chemical shifts of the nonexchangeable protons upon the addition of up to three potassiums per DNA. This DNA forms a basket type structure and exhibits negligible sensitivity to the presence of potassium in the millimolar concentration range. Higher potassium concentrations, tens to hundreds of millimolar, have been shown to alter some features of a dimer crossover structure but not the structural type (*34*). The results on the titration of d(GGGGTTTTGGGG), not shown, were essentially the same as that of the 20mer in that the sample forms a well-defined structure in the presence of sodium alone and that the addition of millimolar potassium induces little change in the spectrum as previously reported (*55*, *65*).

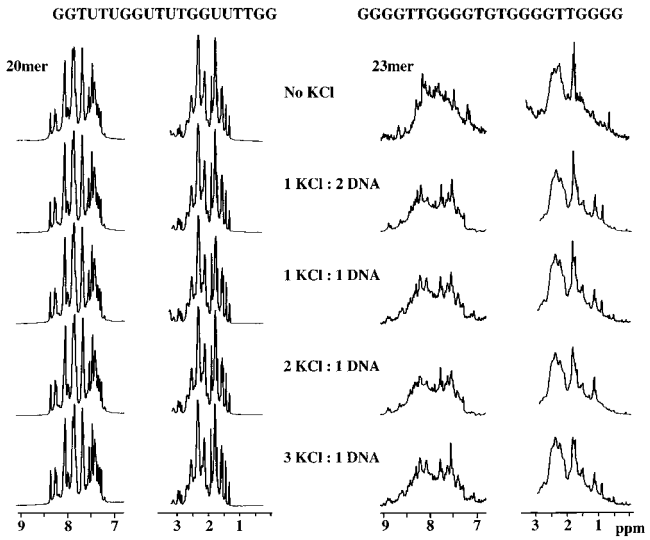

FIGURE 4: One-dimensional spectra obtained during the titrations of the 20mer and 23mer samples. The ratio of DNA to potassium is indicated for each step of the titration.

DNAs Appear To Fall into Three Distinct Categories or Classes. The first class includes the DNAs, such as the 13mer and 14mer, which do not form single, well-defined structures either in the presence of sodium or in the presence of sodium and potassium. The second class includes those DNAs, such as the 15mer, which do form a single, well-defined structure which is of the chair type and do so only in the presence of potassium but not in the presence of sodium alone. The third class includes those DNAs, such as the 20mer, the dimer of d(GGGGTTTTGGGG), and several other examples previously documented (*19*, *57*, *59*, *60*, *66*), which form a single, well-defined structure in the presence of sodium alone and which do not exhibit much sensitivity to the presence of potassium.

The results on the 14mer suggest that a loop of two residues is too short to make the "top" loop of a chair structure as the number of residues in this loop is the difference between the 14mer and 15mer. The results on the 13mer indicate that one residue is not enough to make the "bottom" loops which is the difference relative to the 15mer. These results are consistent with our preliminary modeling studies which indicated that three residues is the minimum needed for the top loop and two residues for the bottom of a chair structure.

These results when combined with those obtained previously (*19*, *57*, *59*, *60*, *66*) indicate that when there are four residues in the top and bottom loops a basket type structure is formed. The results also indicate that when there are three residues in the top loop and two residues in the bottom loops that a chair type structure can be formed but only in the presence of potassium. To examine these tentative rules, further additional loop sequences were investigated.

Effect of Loop Size on Potassium Requirement. The results of the titration of the 16mer, shown in Figure 5, show that this DNA forms multiple structures in the presence of sodium alone. The distribution of chemical shifts suggests that this is a different distribution of structures than that formed by the 13mer, 14mer, and 15mer DNAs in the presence of sodium alone. In the presence of a single potassium per DNA, a different mixture of structures is found. The addition of

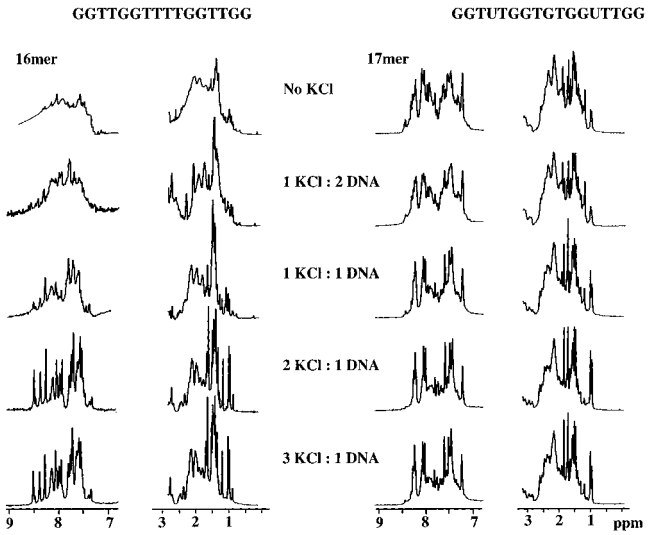

FIGURE 5: One-dimensional spectra obtained during the titrations of the 16mer and 17mer samples. The ratio of DNA to potassium is indicated for each step of the titration.

two potassiums per DNA leads to a single structural form, and the further addition of potassium induces, at most, small changes in the chemical shifts of this DNA.

The structure of the 16mer, in the presence of two potassiums per DNA, is of the chair form. The structure was determined by the methods used to determine the structure of the 15mer. It has been previously shown that the binding sites of manganese will be near all of the *anti*-dG residues of the quartets of a chair type structure, whereas the manganese binding sites are near half of the *anti*-dG residues of the quartets of basket type structures (*48*, *49*). The results of the manganese titration of the 16mer, shown in Figure 6, indicate a chair structure in the presence of potassium as the addition of manganese broadens the resonances of all of the *anti*-G resonances. Thus, it appears that there can be four residues in the top loop in both chair and basket type structures.

The 17mer was examined so as to have a DNA with three residues in each of the three loops. The spectra obtained during the titration of this sample are shown in Figure 5 and indicate that the presence of one potassium induces changes in the chemical shifts. The addition of more than one potassium per DNA does not induce further changes in the chemical shifts. Part of the NOESY spectrum of the 17mer in the presence of potassium is shown in Figure 7. The analysis of the NOE connectivities indicated a chair structure. Further evidence for the presence of a chair structure is given by the results of the manganese titration shown in Figure 7. The addition of manganese broadens all of the resonances of the *anti*-dG residues in the quartets which is an attribute of chair structures (*48*, *49*).

The 20mer was examined as this molecule has four residues in each of the three loops. Part of the NOESY spectrum of the 20mer in the presence of potassium and sodium is shown in Figure 8. The analysis of the NOE connectivities indicated that this DNA forms a crossover or basket structure in the presence of sodium alone and in the presence of potassium and sodium. Evidence for the presence of a crossover type structure is further supported by the results of the manganese titration shown in Figure 8. The addition of manganese broadens only half of the resonances

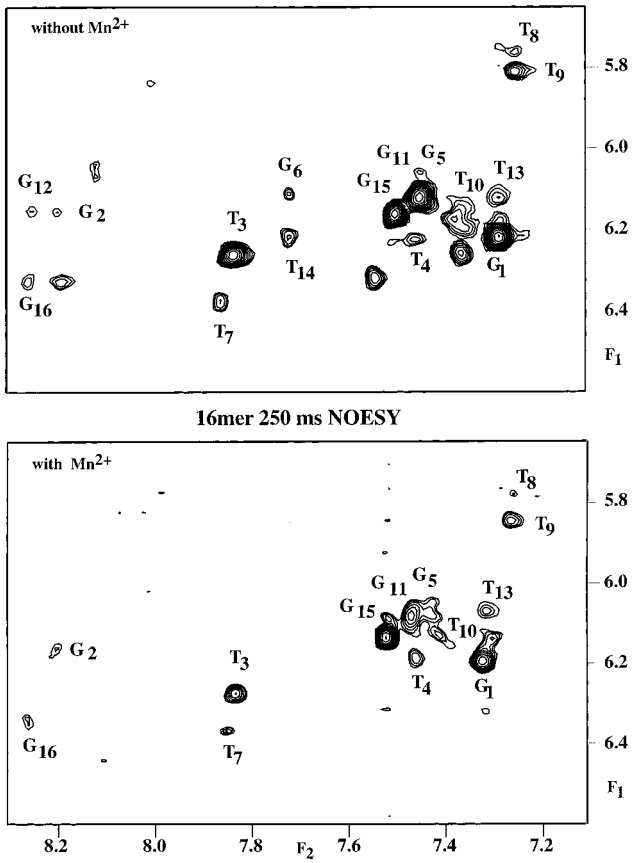

FIGURE 6: 250-ms mixing time 500-MHz NOESY spectrum of the 16mer obtained with the sample in the absence, top, and presence, bottom, of manganese.

of the *anti*-dG residues in the quartets which is an attribute of crossover structures (*48*, *49*).

Effect of the Number of Quartets on Potassium Requirement. The 23mer DNA, whose sequence is shown in Figure 2, has runs of four dG residues and the 15mer has runs of two dG residues, while both have the same loop regions. The potassium titration of the 23mer, shown in Figure 4, indicated that the structure of this DNA is affected by the addition of potassium, but it does not form a single structural form in the presence of potassium. It appears that this DNA forms a number of different structures as indicated by the lack of well-resolved signals in any spectral region. The same results were obtained by heating the 23mer to temperatures up to the boiling point of water and annealing at various rates. This indicates that with runs of four dG residues neither a single chair nor basket structure is formed with three residues in the loops, and the free energies of the two types of structures may be about equal for this sequence. Prior studies have shown that having runs of four dG quartets and four dT residues in each of the three loops gives rise to a basket type structure in the absence of potassium when there are four dG quartets (*58*, *61*). The structure of the crossover type with three dG quartets and four residues in each loop does not require the presence of potassium to form (*59*, *60*).

Energy Minimization and Positions of the Potassium Sites. To examine the structural basis for these specific potassium effects, modeling of the interactions of potassium with the 15mer and 12mer dimers was carried out. In these models the previously determined structures of the DNAs were held fixed and the optimum binding positions of potassium and

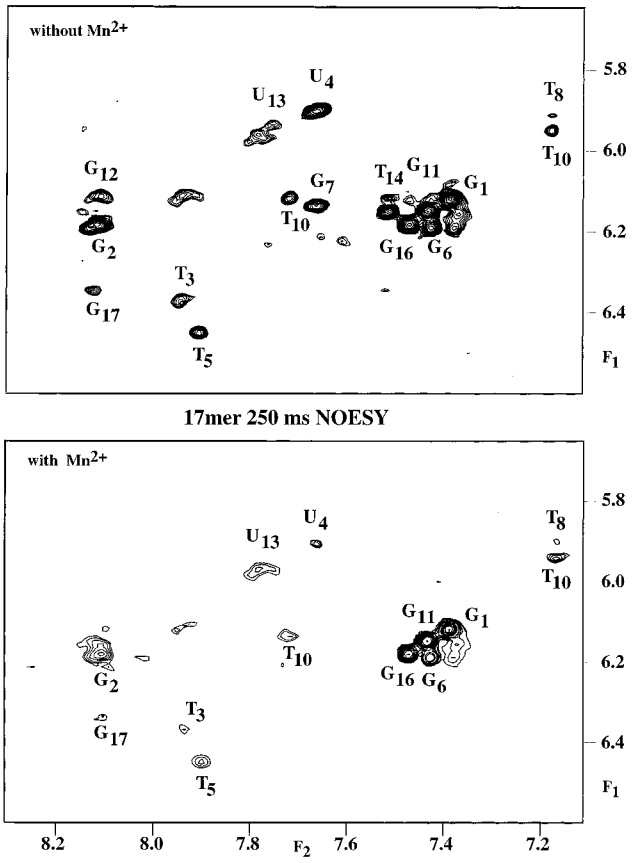

FIGURE 7: 250-ms mixing time 500-MHz NOESY spectrum of the 17mer obtained with the sample in the absence, top, and presence, bottom, of manganese.

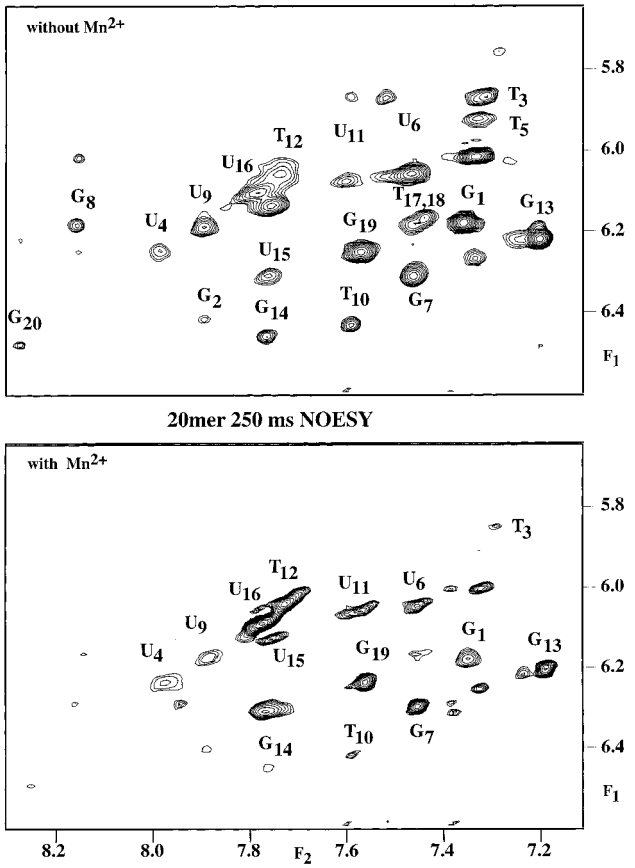

FIGURE 8: 250-ms mixing time 500-MHz NOESY spectrum of the 20mer obtained with the sample in the absence, top, and presence, bottom, of manganese.

sodium determined. The aim was to determine whether there are low-energy sites that are consistent with the results that indicate that the size and sequence of the loops are key determinants of the effects of potassium.

The energies and positions of the sodium and potassium binding sites have been calculated for the chair type structure of the 15mer and the basket type structure of the d(GGGGTTTTGGGG) dimer, and comparably refined structures are available for both (*49*). We have shown that the predicted and experimentally determined binding sites of manganese, for both of these quadruplex DNAs, are essentially identical (*49*). The positions of the manganese binding sites were determined by the enhanced NMR relaxation induced by the binding of the paramagnetic manganese. A similar approach has been used to model the sodium and potassium binding sites of a quadruplex DNA (*62, 63*).

The minimum energy potassium binding sites of the 15mer were found to be between the loops and the quartets as shown in Figure 9. The lowest energy potassium binding sites are close enough to interact with five or six oxygens each. The binding to the top loop and adjacent quartet allows the potassium to be within 3.5 Å of three oxygens. The bottom loop and adjacent quartet appear to form a fairly symmetric binding site with the potassium within 3.5 Å of six oxygens. This modeling suggests that the stronger binding site is between the bottom quartet and adjacent loop as it allows for interaction of the potassium with more oxygens though the calculated energies of the two sites are quite similar. A preliminary structure determination of the one-to-one complex of the 15mer and potassium indicates that the stronger site is the one involving the bottom loop.

Effect of Purines in the Loops on Structure and Potassium Requirement. The modeling also predicts that the substitution of a purine for a thymine in any of the loop positions, other than position 8, will disrupt the binding of potassium by both steric effects and the lack of a suitable carbonyl group ligand. The purine residues at these positions may also have unfavorable interactions with other residues of the DNA. To test this prediction 15mers that contained a dA at position 3, 4, 7, or 9 were titrated with potassium with the results shown in Figure 10. It was found that none of these DNAs exhibited a well-defined structure in the presence of sodium alone. The addition of potassium caused little or no change in the spectral properties of any of these DNAs. These results show that both the sequences as well as the lengths of the loops are determinants of the potassium sensitivity and structures. These results are also consistent with prior studies that showed that the presence of dA in telomeric sequences stabilized the structures in the presence of sodium but not in the presence of potassium (*67*).

Comparison with Cation Binding to Other DNAs. The calculations did not show the presence of a local energy minimum for a potassium binding site between the quartets of the 15mer. Placing a potassium between the quartets at the start of the minimization procedure did not change the final, energy-minimized position. A local energy minimum between the quartets of the dimer of d(GGGGTTTTGGGG) could not be found. There was no local energy minimum between the quartets as the position of minimum energy

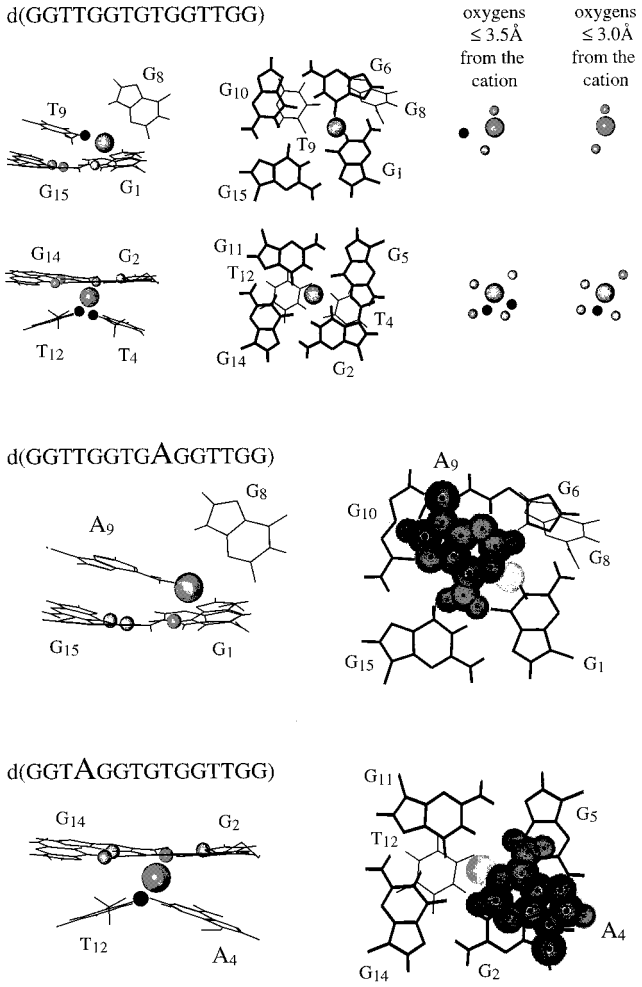

FIGURE 9: Top: Positions of potassium in complexes with the 15mer DNA. The potassium ions are indicated by the spheres which are the size of the van der Waals radius of potassium. From left to right the depictions are of the structures from a "side" view and the "top" view, and selected DNA residues are labeled. The oxygens are depicted as spheres with those "above" the plane in gray and those "below" the plane in black in these two columns. The depictions on the right show just the oxygens that are within either 3.5 or 3 Å of the potassium or sodium, as indicated, with those "above" the plane in gray and those "below" the plane in black. One of the oxygens within 3.5 Å of the potassium in the top loop−top quartet binding site of the 15mer is obscured by the potassium in the second column from the right. Bottom: Calculated positions of the adenines at positions 9 and 4. The depiction on the left shows the residues in stick format, and on the right the adenines are shown in CPK mode to illustrate the steric interactions.

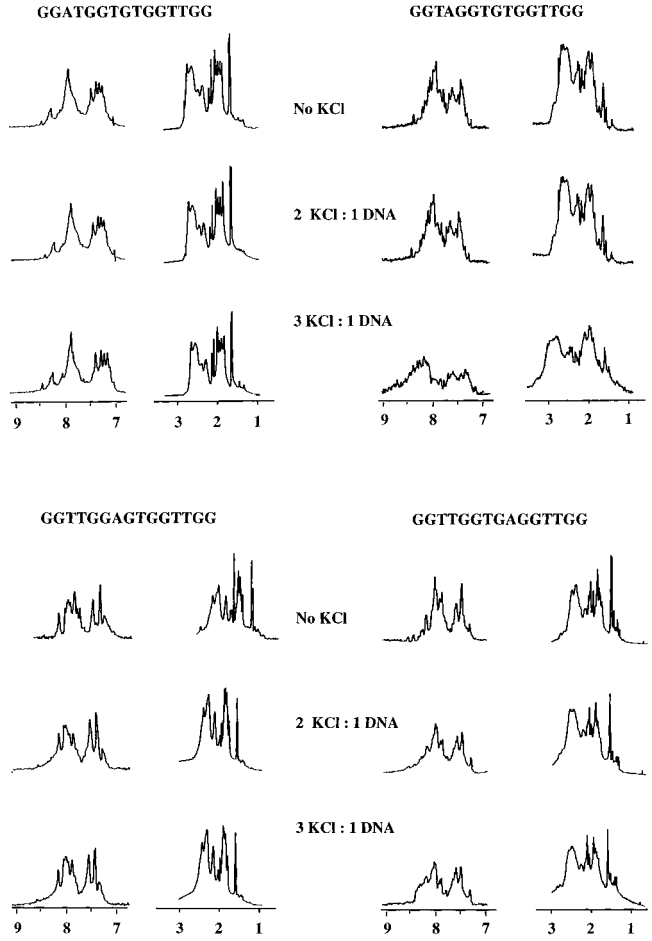

FIGURE 10: One-dimensional spectra obtained during the titrations of the 15mer samples with dA in position 3, 4, 7, or 9. The ratio of DNA to potassium is indicated for each step of the titration.

between the quartets depended on the starting position that was also between the quartets. In both of these cases the energies of the potassium between the quartets were 1 order of magnitude less favorable than the quartet−loop binding sites of the 15mer.

For the d(GGGGTTTTGGGG) dimer the contacts between potassium and the oxygens of the terminal quartet and adjacent loop residues are not nearly as favorable as is the case for the 15mer. The best-calculated site has only one oxygen within 3 Å of the potassium. The energy obtained in the minimization of this complex is quite unfavorable being about 1000 times less favorable than the energy found for the most favorable sites in the 15mer case. These minimization results indicate that potassium does not bind with high affinity to the dimer. This is consistent with the experimental results on potassium binding to a basket type structure which showed relatively weak, multimillimolar binding constant, binding (*34*).

The energies of the crystallographically determined binding sites of sodium to parallel stranded quadruplex DNA (*52*) were also calculated by the same methods for comparison purposes and to test the calculation procedure. The binding sites of sodium between nonterminal quartets have each potassium within 3.5 Å of a total of eight dG O6, and the four dG O6 that are within 3.0 Å of the potassium are all in the same quartet as shown in Figure 9. The calculated energy for the binding of sodium to the parallel stranded quadruplex is comparable to those of the 15mer being about 25−40% less favorable. This indicates that the binding energies of sodium between nonterminal quartets of parallel stranded quadruplex DNA and those of a potassium between a quartet and loop residues of a chair structure are the same within the accuracy of this calculation procedure. Sodium binding between the quartets of a parallel strand quadruplex appears to be much more energetically favorable than that of potassium between the quartets of basket or chair type quadruplex DNAs. This appears to be due to the smaller size of sodium and the larger spacing between the dG O6 oxygens in a parallel strand quadruplex relative to that in the chair or basket forms. The binding site of sodium to the terminal quartet of the parallel stranded quadruplex in the crystal state is quite different from that of the nonterminal sites, and the binding at the termini may be affected by crystal packing.

It may be possible to form a basket or chair type structure with a potassium positioned between two quartets. The structure with a potassium in such a position would have to be distinct from the structures that have been determined so far. No attempt has been made to model structures with potassium between the quartets of chair or basket type structures that allow changing the structures of the DNAs. Energetically favorable binding of potassium would apparently require increasing the distances between the O6 both within each quartet and between adjacent quartets. This might be accomplished by having the quartet dG residues significantly nonplanar.

Outline of Rules for Potassium Requirement. Crossover and parallel stranded structures can be formed in the presence of sodium alone, and the addition of potassium induces small structural changes without altering the type of quadruplex structure present. Potassium binding is required for chair type structures to form, and potassium binds to the terminal quartets and the adjacent loop residues. When the DNA can form a structure with two or three residues in the bottom loops, the DNA will bind potassium to the loops and the adjacent quartet. The binding of a single potassium is sufficient to give rise to a chair type structure. Chair structures with four residues in the top loop are possible as long as the bottom loops have two residues. The case of four residues in the top loop and three in the bottom loops has not yet been investigated, but it is likely to form a chair structure in the presence of potassium. When there are four residues in the bottom loops, a basket structure is formed in the presence and absence of potassium. Last, when there are three or four quartets a basket structure can be formed in the presence of sodium alone, but a mixture of structures is observed in the presence of potassium. When the loops have two or fewer residues, then the DNAs appear to form a mixture of parallel stranded quadruplex structures. This model predicts that the presence of a purine, in most of the loop positions, will disrupt the potassium binding, and chair type structures will not be found.

The potassium interactions with the chair type structures of the 15mer, 16mer, and 17mer show that a diversity of binding modes are possible. The 15mer sequentially binds two potassiums with the binding of the first one being sufficient to bring about a chair type structure. The 16mer also binds two potassiums but does so cooperatively, and both are needed to bring about a chair structure. The 17mer binds a single potassium, and the binding of the single potassium is sufficient for a chair structure to be formed.

On the basis of these results, it appears that with shorter loops one or two potassium ions are required to form a quadruplex structure of the "edge" or "chair" type. When the length of each loop contains four residues, there is apparently no requirement for the presence of potassium and a "crossover" or "basket" type structure is formed. The presence of purines in the loops can apparently preclude the potassium binding by steric exclusion. Structures with three or four quartets are also of the basket or crossover type even with the shorter loops. Recent solution-state NMR results indicate that potassium binds to the loop residues of an edge type quadruplex formed of head-to-tail dimers of d(GGGCTTTTGGGC) and that sodium can bind between the quartets (*62*, *63*). The addition of potassium to the DNA quadruplex in the presence of sodium alters the conformational features of the loops formed of the four dT residues of this quadruplex but not the type of quadruplex structure (*62*). Sodium may also bind between the quartets formed by dimers of d(GGGCTTTTGGGC) (*63*). Similarly, the binding of potassium alters the loop structure of the quadruplex formed by d(GGGCTTTTGGGC) but not the type of quadruplex structure (*63*).

There are some sequences for which these rules make ambiguous predictions. Some naturally occurring telomere DNAs, for example, seem to have sequences which may allow adopting both chair and basket type structures since these rules, by themselves, do not predict whether a particular dG residue will be involved in a loop or in a quartet. Additional studies, analogous to those described here, should be able to determine the importance of the sequences of the loop regions as well as the rules for determining when dG residues will be involved in larger loops or in more quartets.

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