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Probing the structure of multi-stranded guanine-rich DNA complexes by Raman spectroscopy and enzymatic degradation

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Abstract

The multi-stranded DNA complexes formed by the oligonucleotides d($T_{15}G_4T_2G_4$), Tel, and d($T_{15}G_{15}$), TG, were examined by nuclease digestion and Raman spectroscopy. Both Tel and TG can aggregate to form structures consisting of multiple, parallel-oriented DNA strands with two independent structural domains. Overall, the structures of the TG and Tel aggregates appear similar. According to the Raman data, the majority of bases are in C2′-endo/anti conformation. The interaction of guanines at the 3′-ends in both complexes stabilizes the complexes and protects them from degradation by exonuclease III. The 5′-extensions remain single-stranded and the thymines are accessible to single-strand-specific nuclease digestion. The extent of enzymatic cleavage at the junction at the 5′ end of the 15 thymines implies a conformational change between this part of the molecule and the guanine-rich region. The differential enzymatic sensitivity of the complexes suggests there are variations in backbone conformations between TG and Tel aggregates. TG aggregates were more resistant to digestion by DNase I, Mung Bean nuclease, and S1 nuclease than Tel complexes. It is proposed that the lower DNase I sensitivity may be partly due to the more stable backbone exhibited by TG than Tel complexes. Structural uniformity along the guanine core of TG is suggested, as there is no indication of structural discontinuities or protected sites in the guanine-rich regions of TG aggregates. The lower extent of digestion by Mung Bean nuclease at the 3′ end implies that these bases are inaccessible to the enzyme. This suggests that there is minimal fraying at the ends, which is consistent with the extreme thermal stability of the TG aggregates.

Keywords: Raman spectroscopy; DNA structures; Nuclease digestion; Telomere; Electrophoresis

Abbreviations: TG, d($T_{15}G_{15}$); Tel, d($T_{15}G_4T_2G_4$); ps, parallel stranded; aps, antiparallel stranded; TBE, 89 mM Tris, 89 mM boric acid, 2 mM EDTA (pH 8.0)

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1. Introduction

Guanosine can self-associate via hydrogen bonds to form guanine–guanine base pairs [1,2] and guanine quartets [3], giving rise to various DNA structures formed by different guanine-rich sequences, for example, telomeric DNA complexes [4–7], G-wires [8], and frayed wires [9]. Recent interest in the structure and thermodynamics of these complexes arises from their biological importance, their potential as targets for therapeutic agents, and their ability to form self-assembling nanostructures.

The most important biological example of guanine-rich sequences are telomeres, which consist of short, simple guanine-rich sequences repeated hundreds or thousands of times at the end of eukaryotic chromosomes. Telomeres are implicated in mechanisms regulating aging and cancer and they are important in the prevention of chromosomal fusion and degradation [10,11]. Mutations in telomeric sequences can delay or block cell division in anaphase by inhibiting chromosome separation [12,13]. Investigations into the factors important in determining the structure and stability of guanine-rich sequences is important to the understanding of the molecular mechanisms underlying these cellular events.

In a previous study, we reported that substitution of guanine residues for the thymine spacers within the telomereic-like sequence $G_4T_2G_4$, alters the physical and chemical properties of the aggregates formed by this oligonucleotide [14]. The interactions stabilizing the structures formed by oligonucleotides with uninterrupted runs of guanine residues such as, $d(T_{15}G_{15})$, (TG), are distinct from the interactions stabilizing complexes formed by oligonucleotides with a telomere-like sequence, $d(T_{15}G_4T_2G_4)$, (Tel). For example, the guanine N7 sites are required for the formation of tetrads by Tel [3], no aggregation is observed after methylation of this site by dimethylsulfate. However, methylation of guanine N7 sites does not impair the aggregation of TG [14]. Although the guanine–guanine interactions in the TG complexes apparently do not involve hydrogen bonding with the N7 sites, the thermal stability of these complexes is extremely high when compared to that of the telomeric DNA complexes [14]. We have proposed that the unusual stability of the aggregates formed by oligonucleotides such as TG may be biologically relevant. Mutation of a non-guanine residue to guanine would result in the formation of a long run of consecutive guanines, which could then form extremely stable complexes that result in the interruption of cell division, especially during anaphase [12,13].

The complexes formed by Tel and TG also exhibit differences in the stoichiometry of the aggregates and preference of cation for stabilization [14]. We have shown that the successively higher states of aggregation of TG resolved by electrophoresis differ from each other by the addition of a single additional strand of the parent oligonucleotide [14]. This differs from the behavior of the Tel complexes, which are composed of one, two, four, or multiples of four copies of the parent oligonucleotide. These and other data imply that the guanine–guanine interactions in the structures formed by TG and Tel differ.

In this report, we have examined the structure of the TG and Tel aggregates by analyzing their sensitivity to enzymatic degradation and by Raman spectroscopy. The positions and intensities of the Raman lines are sensitive to the main-chain conformation; therefore, we use this technique to diagnose the global structures of these DNA complexes. Because of the sensitivity of enzyme activity to the structure of the substrate, it is possible to deduce information concerning the structure of the substrate by analyzing the relative rates of enzymatic degradation. This is best done in comparison with another, known structure and by employing a range of different enzymes. We have investigated the susceptibility of TG and Tel aggregates to degradation by four nucleases, using single and double stranded DNA as structural standards. Our results reveal that the aggregates of both TG and Tel have parallel strand orientation. And that despite the similarities of the covalent structure of the parent oligonucleotides, TG complexes are more resistant to enzymatic cleavage than Tel complexes implying there are struc-

tural differences between these two aggregates. Both of these multistranded structures are more resistant as well as than the normal double-stranded and single-stranded DNA.

2. Material and methods

2.1. DNA preparation

The oligonucleotides were purchased from the Hospital for Sick Children Biotechnology Service Centre, Toronto. The lyophilized, deprotected, cartridge-purified oligonucleotide were dissolved in 89 mM Tris, 89 mM Boric acid, 2 mM EDTA, pH 8.0 (TBE).

2.2. Raman spectroscopy

Raman spectra were excited with the 514.5-nm line of an argon-ion laser; the radiant power on the sample was 50 mW. The spectra were collected on a SPEX model 1877, 0.6-m Triple Spectrometer with a liquid nitrogen cooled CCD detector (Princeton Instrument Co.). For a typical experiment, 50 l-min exposures were accumulated and averaged.

The aggregate-forming oligonucleotides, TG and Tel were dissolved in TBE and incubated with either 1 M $MgCl_2$ at 90°C for 30 min or 1 M KCl at 90°C for 5 min prior to acquisition of Raman spectra.

2.3. Nuclease digestion

The DNA oligomers were labeled with ^{32}P using [γ-^{32}P]ATP and polynucleotide kinase and subsequently desalted using a P-6 Bio-Spin Chromatography column (Bio-Rad, Inc.). The aggregates of TG and Tel were prepared with 200 mM $MgCl_2$ at 90°C for 30 min or 200 mM KCl at 90°C for 5 min. The heated samples were then slowly cooled to room temperature before use. Single stranded $d(T_{15}N_{15})$ and double stranded $(T_{15}N_{15}).d(A_{15}N_{15})$ were used as controls.

We followed two protocols in the nuclease digestion experiments. To mimic the condition in extracellular fluid, all digestions were carried out in Hank's solution at 37°C (5 mM KCl, 0.3 mM KH_2PO_4, 138 mM NaCl, 4.0 mM $NaHCO_3$, 0.3 mM Na_2HPO_4, 5.6 mM D-glucose). For one set of experiments we used extremely high concentrations of the enzyme to degrade the aggregates. One-minute incubations at 37°C with 249 U of Exonuclease III (Exo III), 332 U of S1 nuclease, or 156 U of Mung Bean nuclease were used. In another series of experiments more moderate amounts of these enzymes were used and the incubation period was varied. Nucleases used in this part of the study were: Exonuclease III (4.98 U/μl in 20 mM $MgCl_2$), DNase I (3 U/μl in 20 mM $MgCl_2$), S1 (6.64 U/μl in 20 mM $MgCl_2$ and 0.1 mM $ZnSO_4$), and Mung Bean nuclease (6.24 U/μl in 20 mM $MgCl_2$ and 0.1 mM $ZnSO_4$). For both of the nuclease digestion experiments, 1 μl of sample was incubated for the desired length of time with 5 μl of a solution containing the enzyme at the appropriate concentration. The reaction was stopped by adding 2 μl of 0.5 M EDTA. The digested products were then examined by electrophoresis on native and denaturing polyacrylamide gels. The native gels were run at 4°C.

3. Results

3.1. Raman spectroscopy

In TBE, in the absence of added cations, TG, and Tel, containing only guanosine and thymidine residues, exhibit similar Raman spectra (Fig. 1). TG complexes formed in the presence of Mg^{2+} and Tel aggregates formed in the presence of K^+ had a prominent peak at 837 cm^{-1} (Fig. 1). This peak is characteristic of B-form DNA with the furanose rings in the C2′-endo conformation [15]. The peaks at 837 cm^{-1} and 1091 cm^{-1} are indicators of the local backbone geometry, and their position implies that the backbone is similar to that of B-DNA [15,16]. The marker band for the phosphate group at 1091 cm^{-1} was employed for normalization of intensities [17]. The spectra of both aggregates exhibited an intense band at 746 cm^{-1} indicating that a majority of thymine residues adopt the C2′-endo/anti conformation (Fig. 1).

Fig. 2 displays the Raman spectral region from

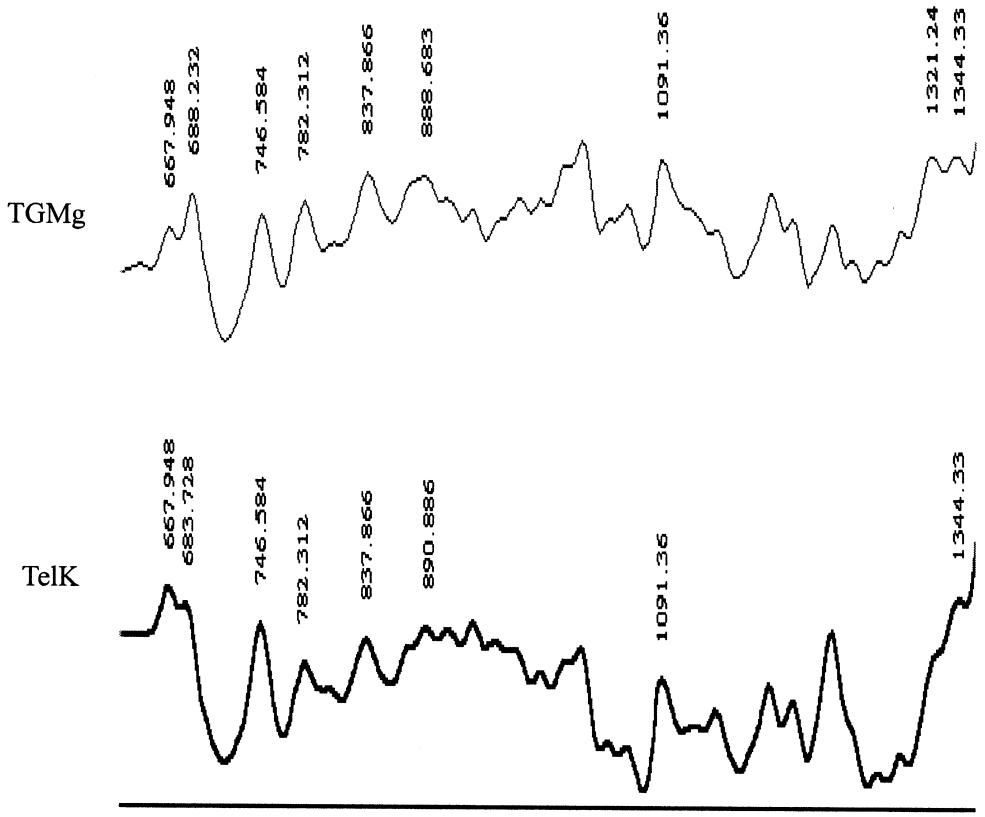

Fig. 1. The Raman spectrum (650–1350 cm^{-1}) of TG aggregates formed in 1 M $MgCl_2$ in TBE and Tel aggregates formed in 1 M KCl in TBE. The data were smoothed by employing a 5% polynomial.

640 to 720 cm^{-1} of single stranded, i.e. non-aggregated, TG and Tel; the spectra of their aggregates are also shown. This spectral region contains marker bands for different sugar conformations of the guanine residues. Parallel strand orientation through intermolecular association via the guanine bases results in a single sugar conformation (C2′-endo/anti), whereas antiparallel strand orientation gives rise to two sugar conformations, C2′-endo/anti and C2′-endo/syn [16]. Before incubation with cations, TG and Tel exhibited a broad asymmetric band in the 670–680 cm^{-1} spectral region (Fig. 2A,C). The broad band of Tel in buffer (Fig. 2C), can be fit by two Gaussian curves, one centered at 666 and the other at 680 cm^{-1}. After heating Tel in the presence of K^+, the broad asymmetric band splits into a band at 668 cm^{-1} arising from the thymine residues and a band at 686 cm^{-1} that is due to guanosine residues in the C2′-endo/anti conformation [16] (Fig. 2D). Similar splitting is observed for TG, (Fig. 2A,B). The frequency of the band we attribute to guanosine in a C2′-endo/anti conformation is 688 cm^{-1} which is somewhat higher than the frequency in Tel, 686 cm^{-1}. However, it is within the experimental uncertainty. The spectra in Fig. 2, imply that the guanosine residues of both Tel and TG adopt the C2′-endo/anti conformation upon aggregation. From this we infer that the DNA strands associate in a parallel orientation [16,18,19].

3.2. Nuclease digestion

We have used nuclease digestion to probe the secondary structures of self-associated species formed by TG and Tel. The extent, rate, and pattern of enzymatic digestion of these aggregates

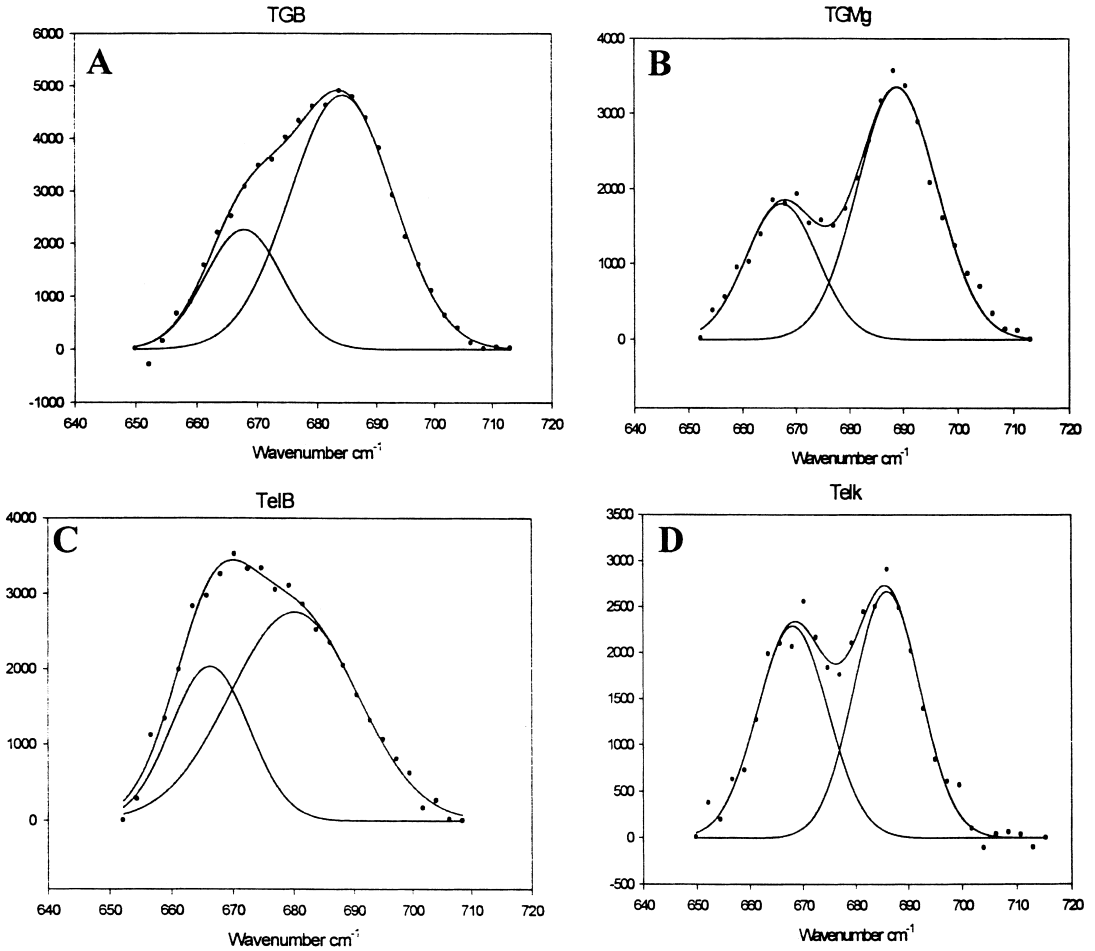

Fig. 2. Raman spectra of TG and Tel aggregates in the region of dG nucleoside conformation markers. Data were fitted with two Gaussian peaks. (A) TG oligonucleotides in TBE with peaks at 667 and 684 cm^{-1}. (B) TG aggregates formed in 1 M $MgCl_2$ in TBE with peaks at 667 and 688 cm^{-1}. (C) Tel oligonucleotides in TBE with peaks at 666 and 680 cm^{-1}. (D) Tel aggregates formed in 1 M KCl in TBE with peaks at 668 and 686 cm^{-1}.

were compared to the behavior of single and double-stranded DNA molecules. The ability of an enzyme to catalyze a reaction depends upon the structure of the substrate. If the substrate adopts a structure incompatible with the substrate recognition and catalysis of a particular enzyme, then it will remain unreacted. Analysis of the differential enzymatic reactivity provides a tool for the structural analysis of the substrate, this is comparable to other chemical methods such as methylation protection and footprinting. We have used four nucleases to probe differences in the structures of the aggregates formed by TG and Tel.

We performed two types of enzymatic digestion experiments, one in which the DNA was reacted with a large amount of enzyme for a short period and a second where the DNA was incubated with the enzyme for periods up to 24 h. As shown in Fig. 3, a 1-min exposure of TG or Tel aggregates to a large amount (249 U) of Exo III nuclease does not lead to any apparent hydrolysis. However, the aggregates are digested by Mung Bean and S1 nucleases, both of which are single-

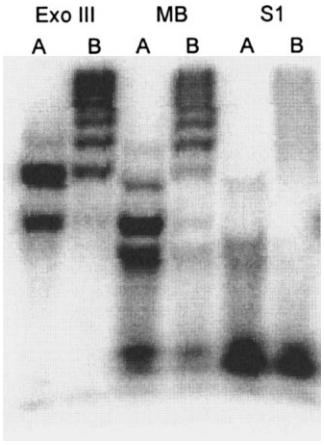

Fig. 3. Digestion with exonuclease III (249 U), Mung Bean (156 U) and S1 endonucleases (332 U) for a 1-min incubation at 37°C. (A) ^{32}P-Tel. (B) ^{32}P-TG.

strand-specific (Fig. 3). Comparison of the relative intensities of the bands demonstrated that the TG aggregates are more resistant than Tel complexes to enzymatic degradation.

The aggregates formed by TG or Tel oligonucleotides are inefficiently cleaved upon extended incubation with Exo III (Fig. 4). During the first 5 min, almost 40% of the double stranded reference DNA was digested while only 1–2% of the TG or Tel aggregates were degraded (data not shown). After 24 h, only 3% of the TG or Tel complexes were degraded compared with 70–80% digestion of double-stranded or single-stranded DNA (data not shown). The incomplete cleavage of double-stranded and single-stranded DNA is due to the phosphate content of Hank's solution which would interfere with the action of the enzymes. Despite this, it is clear that extent of digestion of the TG and Tel complexes by Exo III is dramatically lower than that of the double-stranded or single-stranded DNA. Although TG and Tel complexes have comparable rates of digestion, the two thymine spacers within the Tel sequence are preferably cut by Exo III nuclease (Fig. 5).

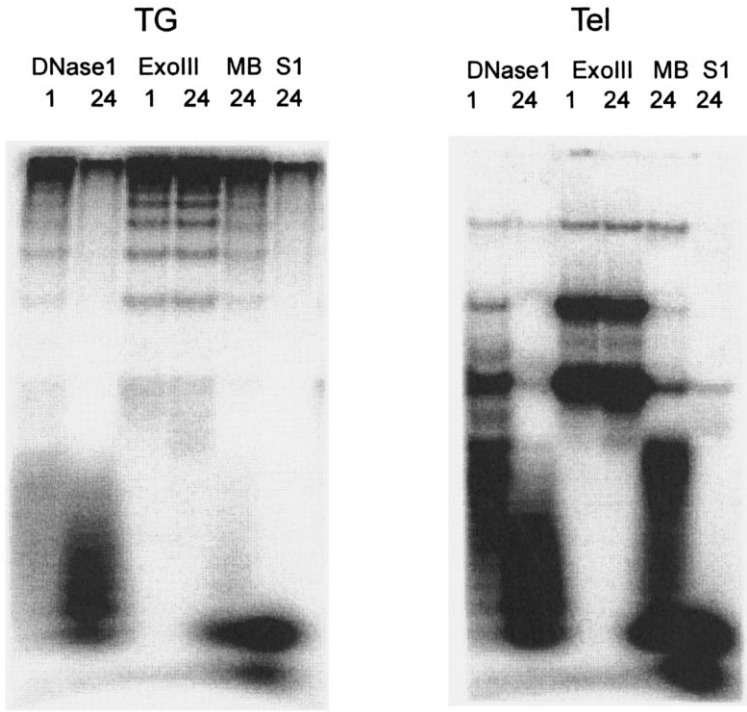

Fig. 4. Native electrophoretic gel (15%) showing the digestion products of TG and Tel complexes. The complexes were digested with DNase I (15 U/sample for 1 and 24 h), Exonuclease III (ExoIII, 24.9 U/sample for 1 and 24 h), Mung Bean nuclease (MB, 31.2 U/sample for 24 h) and S1 (33.2 U/sample for 24 h). Note that neither sample exhibited any digestion after 1 hr incubation with Exo, so the gel patterns reflect the original samples of TG and Tel complexes.

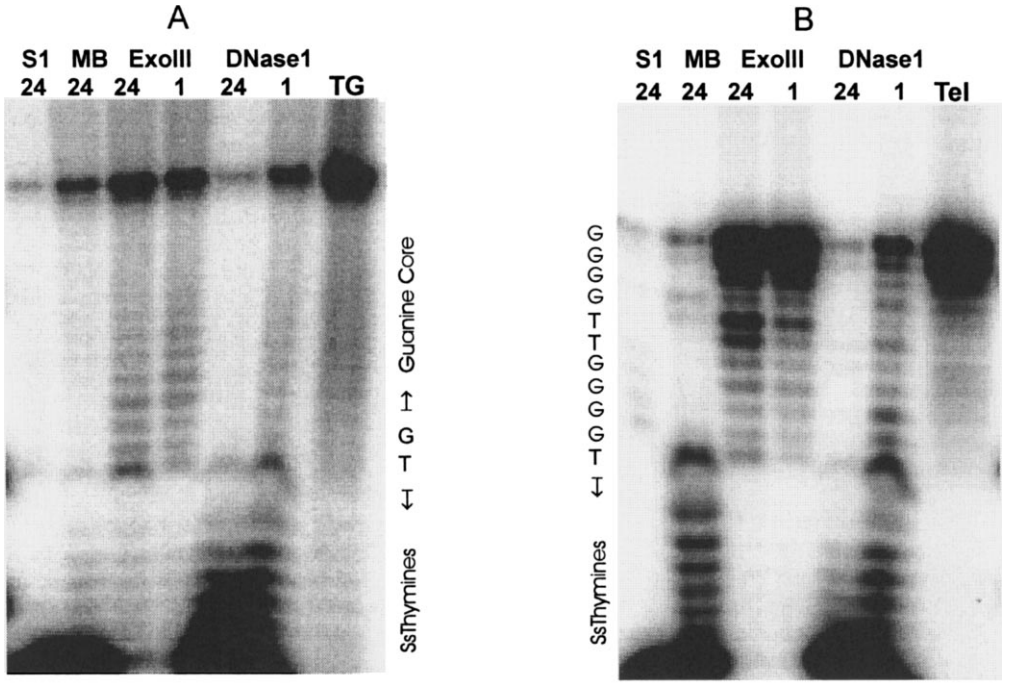

Fig. 5. Twenty percent denaturing gel showing the digestion products of (A) TG and (B) Tel complexes. The complexes were digested with DNase 1 (15 U/sample for 1 and 24 h), Exonuclease III (Exo III, 24.9 U/sample for 1 and 24 h), Mung Bean (MB) nuclease (31.2 U/sample for 24 h) and S1 (33.2 U/sample for 24 h).

TG and Tel aggregates were digested by DNase I, with only 20–30% aggregates remaining at the end of a 24-h incubation (Fig. 4). However, both of the aggregated species were more resistant than double or single stranded DNA toward this enzyme (data not shown). The single-stranded thymines are more extensively degraded than the guanines for both TG and Tel aggregates (Fig. 5). Examination of the rate of digestion by DNase I shows that the TG aggregates are more resistant to cleavage than the Tel complexes, especially during the first hour of digestion (Fig. 4). Although more cutting was observed in the guanine-rich region of the Tel complexes, in either aggregate, this region was inefficiently digested (Fig. 5).

Figs. 4 and 6, show that the single-stranded thymine residues at the 5′ end of both the TG and Tel aggregates are accessible to digestion by the single-strand-specific enzymes Mung Bean nuclease and S1 nuclease. At the end of a 24-h incubation, 40–50% degradation was observed in TG aggregates and 80–90% in Tel complexes (data not shown). The difference in degradation rate was more dramatic during the first hour of incubation (Fig. 6). The data show that Mung Bean nuclease apparently digests Tel complexes from the 3′-end, where they are stabilized by guanine–guanine interactions, and proceeds towards the 5′-end. S1 and Mung Bean nucleases rarely cut within the G-run region of the TG complexes. This argues against the existence of significant number of guanine bases looped out from the central G-core of the aggregates (Fig. 7a). For both types of aggregates, hypersensitive cleavage sites at the junctions between the guanine and thymine residues were observed for the single-strand-specific nucleases (Fig. 6).

4. Discussion

We have previously shown that the formation of stable multistranded aggregates by Tel, $d(T_{15}G_4T_2G_4)$, is promoted by potassium ions but not by magnesium ions [14]. Modification of the sequence within the guanine-rich region to

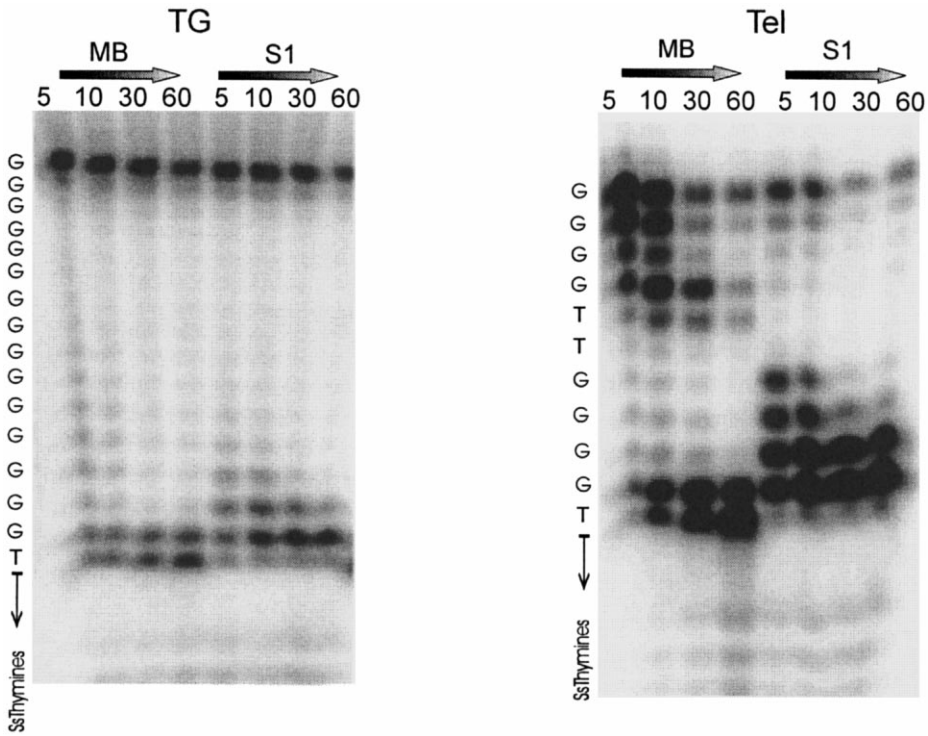

Fig. 6. Twenty percent denaturing gel showing the digestion products of TG and Tel complexes. The complexes were exposed to Mung Bean (MB) nuclease (31.2 U/sample for 5, 10, 30, and 60 min) and S1 nuclease (33.2 U/sample for 5, 10, 30, and 60 min).

$d(T_{15}G_{15})$ (TG), one observes the formation of stable multistranded aggregates in the presence of either potassium or magnesium ions [14]. We showed that the complexes formed by TG in the presence of Mg^{2+} have chemical and physical properties distinct from those of Tel aggregates. We anticipated that the origin of these distinct properties would lie in structural differences between TG and Tel aggregates. However, the Raman data presented here show that the sugar conformation of the TG and Tel aggregates are similar. Both types of aggregates consist of multiple, parallel DNA strands, in which the sugars have B conformation and the majority of bases are in the C2′-endo/anti conformation.

Although telomeric DNA sequences can form either parallel or anti-parallel multi-stranded complexes, their ability to adopt different conformations is determined by cation conditions and the number of thymine spacers within the telomeric sequences [4]. Studies using the telomeric sequence of *Oxytricha Nova* showed that it could form a parallel-stranded complex at high concentrations of either sodium or potassium ions while anti-parallel species are prevalent at low cation concentrations. Since the salt concentrations used in the present studies were higher than those reported necessary to form parallel stranded species [16], it is expected that parallel strand arrangement would be favored. One way to obtain an anti-parallel structure involves the interaction of two hairpin structures. For a tetraplex, this requires that the thymine spacers within the telomeric sequence loop out of the helix allowing the oligonucleotide chain to fold-back on itself and the guanine bases to engage in tetraplex interactions [4]. A minimum of two thymine spacers is needed to form a stable fold-back structure [4]. The absence of a thymine spacer in the TG oligonucleotide would appear to eliminate the possibility of forming antiparallel fold-back structures, implying that the strands in

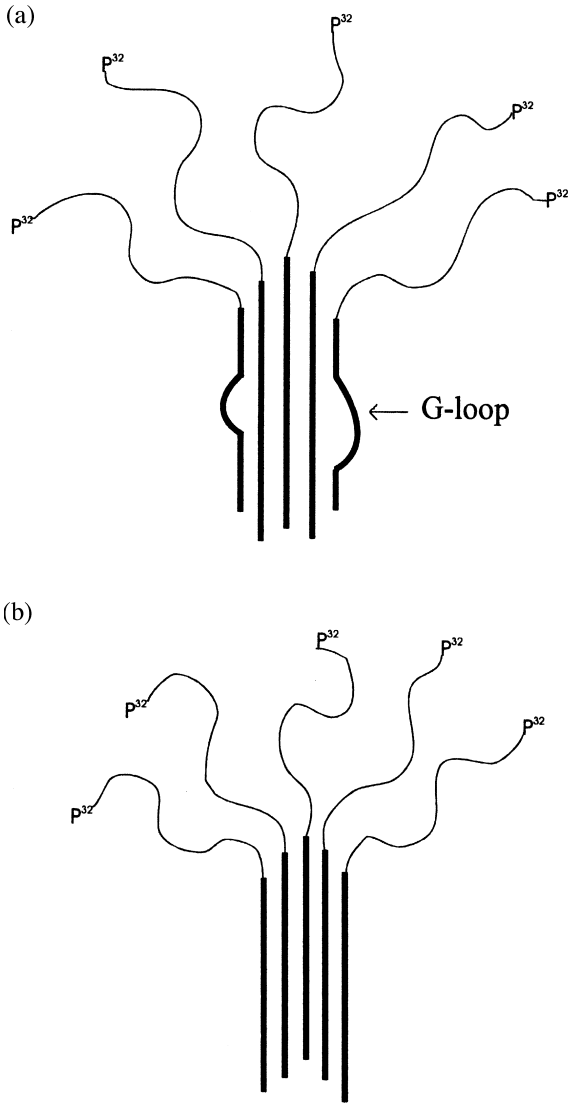

Fig. 7. A schematic presentation of TG complex which is composed of five TG monomers. The thick line (—) represents the regions consisting of guanine residues and the thin line (—) for that consisting of thymine residues. These sequences are ^{32}P-labelled at the 5′-end. The picture shows that the TG complex is stabilized via the guanine region with the thymine residues freely hanging at the 5′position. (A) G-looped model: parts of the guanine bases loop out from the central G-core forming G-loops. (B) Homogeneous unfolded model: The G-rich region is unfolded with all the bases engaging in stabilization interactions.

TG aggregates must be oriented in a parallel manner. However, the guanine–guanine interactions in the parallel form of TG aggregates are not necessarily equivalent to those of the Tel complexes. This dissimilarity is supported by the differential behavior of the N7 sites of the guanine bases to methylation and nuclease digestion as discussed below.

In the present study, we demonstrated that TG and Tel aggregates have different nuclease sensitivities. Thus, although the Raman data imply that the aggregated complexes have similar overall structures, the difference in their nuclease sensitivity implies the existence of structural variations between them. Although TG complexes are stabilized by guanine–guanine interactions, we have previously shown that their properties differ from those of standard guanine tetraplex in telomeric (Tel) complexes [14]. The most striking chemical difference we have observed is that methylation of the N7 group of guanine does not inhibit aggregate formation by TG. This is distinct from the behavior observed for telomeric sequences for which N7 methylation abolishes aggregation, [5,6,20,21]. In standard tetraplexes, the N7 sites of guanines are involved in the formation of the exterior hydrogen bonding of guanine tetraplex. Somewhat unexpectedly, we found that not only would TG form aggregates upon methylation but these aggregates are also more stable than the complexes formed by unmethylated Tel. This result led us to propose that the TG and Tel complexes involve different types of guanine–guanine interactions; however, the details of the difference remain unknown.

In this work, we have investigated the relative sensitivity of Tel and TG to nuclease digestion. Our results reveal that the stabilization force via the guanine–guanine interactions at the 3′-end of TG and Tel aggregates inhibits degradation by Exo III nuclease. In contrast to double- and single-stranded DNA, TG and Tel complexes are very resistant to degradation by Exo III, with either incubation protocol, high dose, short exposure time or low dose, long exposure time. These results imply that the 3′-ends of these multistranded DNA complexes are not recognized by Exo III and hence are dissimilar from more standard DNA structures.

Because the reactivity of DNase I is well characterized, it is commonly used as a probe to assess the conformational changes in DNA [22].

This enzyme binds to the minor groove of the B DNA double helix forming contacts with the phosphates that are opposite each other on the two strands. DNase I catalyzes the hydrolysis of the 3′O-P phosphodiester bond in single and double stranded DNA by nucleophilic attack. Its reactivity is strongly influenced by the size of the helix groove and the spacing between the phosphates across the minor groove [22], but not base-pair arrangement. The cleavage rate is sensitive to local, sequence-dependent conformational variations [22], for example, local helical twist [23]. Cleavage is inhibited when the disposition of the phosphates no longer resembles that of B-DNA [22].

As shown in Fig. 5, most of the DNase I cutting for both TG and Tel aggregates occurred in the 5′ thymine residues rather than within the guanine residues at the 3′-end. The guanine–guanine interactions in both TG and Tel complexes seem to significantly reduce the cutting rate of DNase I. This reflects that the minor grooves of both TG and Tel complexes are different from that of B-DNA. This is consistent with our finding that TG and Tel complexes are parallel multi-stranded complexes; therefore, the groove width, radial symmetry and accessibility of the phosphates will differ from those of double stranded molecules. This is reflected in their relatively lower rate of cleavage by DNase I. Neither hypersensitive DNase I cleavage sites, nor any protected sites were found within the guanine cores.

The 5′ non-guanine regions of telomeric sequences and molecules like TG have been shown to protrude from the aggregated structures as single stranded arms [7,9,14]. Consistent with this structure we found that the 5′-thymine extensions of both complexes were accessible to digestion by single-stranded-specific nucleases.

S1 and Mung Bean nucleases both preferentially cut at the junctions between the guanine cores and the single stranded thymines of the TG and Tel aggregates. Other enzymatic studies have reported preferred cleavage at conformational junctions, for example between right-handed B-DNA and left-handed Z-DNA [30], and between double stranded and triple stranded DNA [31]. The hypersensitive sites at the junctions of Tel and TG aggregates suggest, not surprisingly, a conformational difference between the 3′ guanines and the 5′ thymine extensions.

The variation in the ability of nucleases to recognize the aggregates as substrates is a manifestation of their structural differences. The finding that TG aggregates are digested by DNase I at a lower rate than the Tel complexes implies that the backbone structures of the aggregates are not the same. Thomas et al. [24] have shown that uninterrupted runs of guanines are very rigid, perhaps the lack of conformational flexibility is responsible for the limited extent of DNase cleavage [25]. The thymine loop linking the two sets of four guanine bases in the telomeric sequence was preferentially cleaved by S1 nuclease (Fig. 6). S1 nuclease recognizes and cleaves exposed phosphodiester bonds in loops more readily than those in a double helix [22]. This enzyme readily cuts bonds close to the 5′-end of a hairpin and the cleavage proceeds rapidly toward the 5′-end [22]. Similar to DNase I, S1 is relatively insensitive to sequence; however, the rate of catalysis depends on the structural details of sugar–phosphate backbone [22]. The higher cutting rate of S1 within the thymine loop of Tel aggregates implies that these phosphodiester groups are more exposed than those of the four adjacent guanines. The data shown in Fig. 6 also revealed that once the thymine loop was nicked, S1 nuclease can proceed along the guanines which are stabilized by guanine tetrads (Fig. 8b). It seems unlikely that this result is a secondary cleavage effect of cutting from the 3′-end. Drew [22] has clearly shown that a phosphate group at the 3′-side of the cleavage site of the substrate is necessary for S1 nuclease to cleave. The absence of a phosphate group at the 3′-end of the Tel aggregates would hinder cutting from this end.

Although S1 and Mung Bean nucleases are single-strand-specific, they can cleave double stranded molecules because of base pair fraying at the ends of double stranded DNA [26–28]. In the present study, we found the 3′-ends of Tel were more susceptible to single-stranded-specific nuclease digestion than duplex DNA (data not

shown). This was not observed for the TG aggregates. Fraying of DNA involves a localized, rapid association and dissociation of the base pairs at the end of the DNA strands. For an enzymatic reaction to proceed, the lifetime of the dissociated state has to be long enough to allow the enzyme to bind. Thus, the greater the extent of fraying, the greater is the chance of enzymatic cleavage. The higher digestion rate of Tel at the 3′-end by single-stranded-specific nucleases may reflect extensive fraying at this end (Fig. 8a).

Under similar experimental conditions, Mung

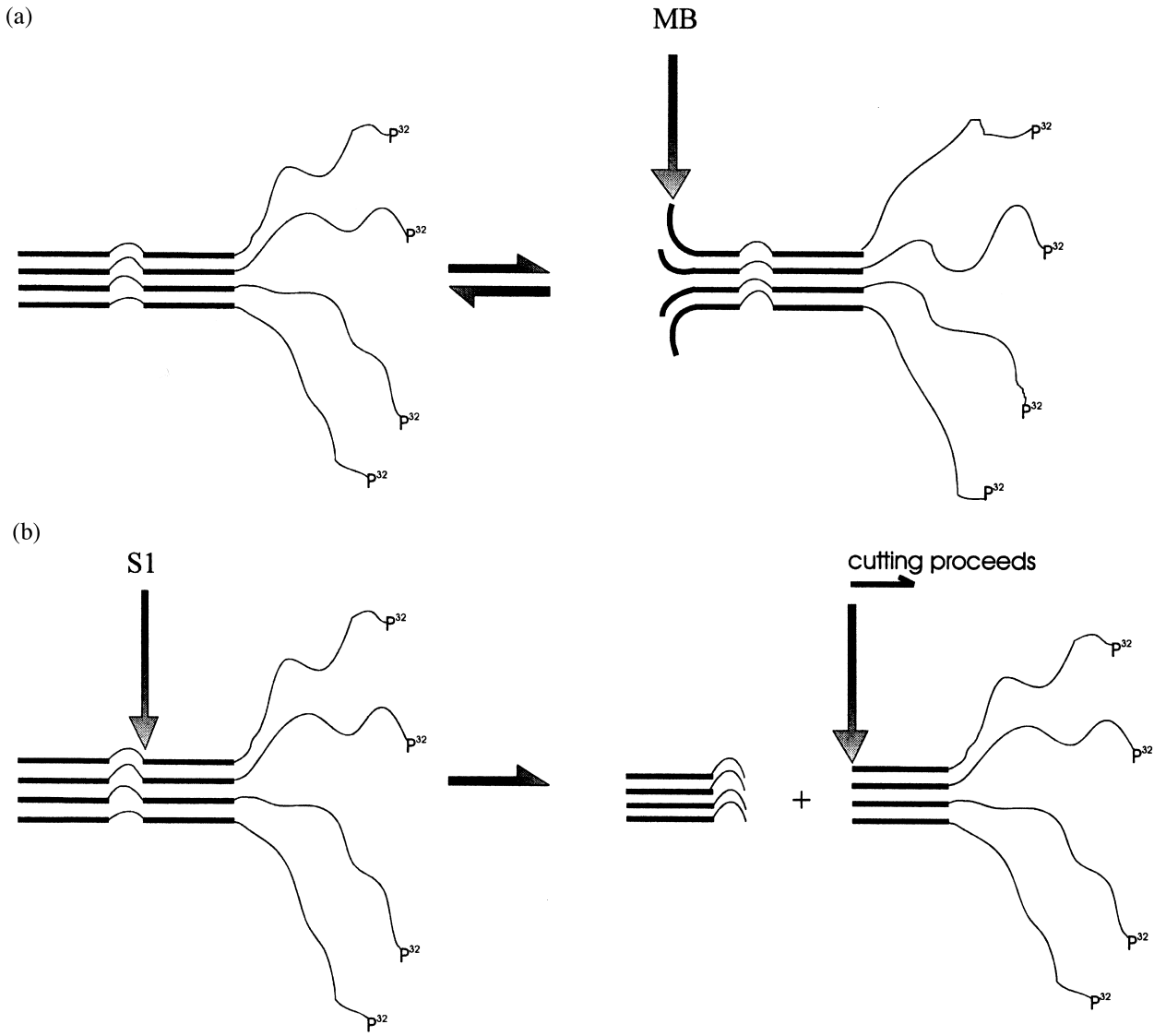

Fig. 8. A schematic presentation of TG complex which is digested by Mung Bean (MB) and S1 nucleases. The thick line (—) represents the regions consisting of guanine residues and the thin line (—) for that consisting of thymine residues. The drawing shows that the TG complex is formed by four Tel monomers and is stabilized via the guanine region. These sequences are ^{32}P-labelled at the 5′-end. (A) MB is shown to cut at the fraying region of the 3′-end. (B) S1 is shown to preferentially cut at the thymine loop and proceed with cleavage in the 5′ direction.

Bean nuclease cleaves more slowly than S1 [29], however, we found that it hydrolyzes the 3′-terminal guanine bases of the Tel aggregates more efficiently than S1 (Fig. 6). This may be due to the fact that S1 preferentially cleaves at positions with a 3′ phosphate [22], there is none at the 3′-end of the Tel aggregates. Mung Bean nuclease apparently does not require a 3′ phosphate, thus cutting can proceed from the 3′-end (Fig. 8a).

We do not detect much single-stranded cutting along the G-run of the TG aggregates. This indicates that there are few single-stranded loops formed along the G-run region. This argues against the G-looped model of the TG aggregates (Fig. 7a) in which some of the guanine bases are looping out from the G-core region and do not form a stabilizing interaction with those along the central G-core. Instead, the data is consistent with a homogeneous G-core structure (Fig. 7b) in which all the guanine bases interact with each other.

The backbone heterogeneity in the guanine-rich region of Tel due to the interspersed thymine residues enhances the reaction of the single-stranded-specific enzymes. Although a certain degree of backbone heterogeneity within the guanines may exist in TG aggregates, no hypersensitive sites within the run of guanines were observed, and overall, these structures are much more stable than the Tel aggregates with respect to cleavage by S1 or Mung Bean nuclease. This finding is consistent with TG and Tel aggregates having different variations of backbone conformations. The absence of fraying implied by the lack of enzymatic cleavage is also consistent with the greater thermal stability of the TG complexes. The resistance of TG aggregates toward cleavage by these enzymes may have the same structural origin as the thermal stability of TG aggregates.

In summary, the multistranded aggregates formed by TG and Tel appear to have structures that are globally similar. Both types of aggregates consist of multiple parallel DNA strands, but they exhibit differences in their stoichiometry and thermal stability. Generally, TG aggregates are quite resistant to nuclease cleavage. The nuclease digestion results imply that the guanines in Tel and TG aggregates do not adopt the same local conformation. There is no evidence of looping out of bases within the guanines of the TG aggregates; all of the guanines are uniformly accessible, or resistant, to cleavage. These data are consistent with the existence of a uniform structure along the guanine core of the TG aggregates (Fig. 8b). The accessibility of the guanines to cleavage by single strand-specific nucleases imply that there is fraying at the 3′ end of the Tel aggregates. However, fraying does not appear to be extensive in the TG aggregates.

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