Structural characterization of DNA and RNA sequences recognized by the gene 5 protein of bacteriophage fd

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The single-stranded DNA sequence d($GT_5G_4CT_4C$) occurs close to the origin of replication within the intergenic region of the viral strand of bacteriophage fd. The RNA analogue of this sequence r($GU_5G_4CU_4C$) forms part of the untranslated leader sequence of the gene 2 mRNA and is specifically bound by the fd gene 5 protein in its role as a translational repressor. The structure of these sequences is likely to have an important role in the control of both DNA replication and RNA translation in the phage. We show that this 16 nt sequence, in both a DNA and an RNA context, can exist in a structured and unstructured form as determined by high-resolution gel filtration and non-denaturing gel electrophoresis. The CD spectrum of the structured form is characteristic of parallel guanine tetraplexes. The structured form of the DNA sequence melts at approx. 47 °C in the presence of Na^+ ions but the structure is stabilized up to 75 °C in the presence of K^+ ions. The RNA structure is more stable than the equivalent DNA structure (melting temperature approx. 62 °C), and its stability is further enhanced in the presence of K^+ ions. Two of the central guanine residues are fully protected from cleavage as determined by dimethyl sulphate protection experiments, whereas methylation interference studies show that methylation of any of the four central guanine residues inhibits structure formation. Our results demonstrate that the structured form of the nucleic acid is mediated through the formation of a guanine-tetraplex core region, in RNA this might be further stabilized by the presence of weaker uracil quartets.

Key words: circular dichroism, dimethyl sulphate footprinting, guanine quartets, single-stranded-DNA-binding proteins, tetraplexes.

INTRODUCTION

Proteins that bind single-stranded DNA constitute an important class of DNA-binding proteins, with a key role in DNA replication, transcription and translation [1]. The gene 5 protein (g5p) of filamentous bacteriophage fd is the most widely studied of the binding proteins for single-stranded DNA [2]. g5p is responsible for the switch from double-stranded to single-stranded viral DNA replication; it binds co-operatively to the single-stranded circular viral DNA to form a large helical nucleoprotein assembly [3] before packaging of the DNA into the virion. The fd g5p also functions as a translational repressor, binding specifically to the leader sequence of the gene 2 mRNA to down-regulate the expression of gene 2 [4,5]. Earlier studies have identified the 16-base RNA sequence r($GU_5G_4CU_4C$) that is specifically recognized by g5p; single-base changes to this sequence can impair the ability of g5p to repress translation [6]; most of these mutations impair RNA binding *in vitro* [7].

The DNA analogue of this sequence, d($GT_5G_4CT_4C$), was also shown to be preferentially bound by g5p; mutations that affect translational repression *in vivo* and RNA binding *in vitro* also impair the ability of g5p to bind the DNA sequence [7]. The 16-base DNA sequence seems to exist as two conformers and it has been suggested that this could arise from G–T base-pair formation; in contrast, only one conformation was observed for the equivalent RNA sequence [7].

Here we investigate the nature of two structural forms of the 16-base DNA sequence d($GT_5G_4CT_4C$) and show that the equivalent RNA sequence r($GU_5G_4CU_4C$) can also exist in two structural forms. On the basis of CD and chemical modification studies, we propose that the structured conformation is mediated through the formation of guanine quartets (G-quartets). We show that in the presence of Na^+ the RNA structure is more stable than the DNA structure, and that both are greatly stabilized in the presence of K^+.

EXPERIMENTAL

Oligonucleotides

The nomenclature and sequence for each oligonucleotide are as follows (2-aminopurine is represented by X):

DNA-6	5′-dTGGGGT-3′
RNA-16	5′-rGUUUUGGGGCUUUUC-3′
DNA-16	5′-dGTTTTTGGGGCTTTTC-3′
DNA-16AP	5′-dGTTTTTGXGGCTTTTC-3′
DNA-16C	5′-dGAAAAGCCCCAAAAAC-3′
DNA-24	5′-dTCCTGTTTTTGGGGCTTTTCTGAT-3′

Synthetic oligonucleotides (HPLC-purified) were purchased from a variety of sources (Cruachem, Oswel and Genosys Custom Biopolymers). Oligonucleotides were 5′ end-labelled with [γ-^{32}P]ATP (ICN) in the presence of T4 polynucleotide kinase (New England Biolabs) as described by Maniatis et al. [8].

Determination of molar absorption coefficients

Molar absorption coefficients of oligonucleotides were determined by digestion to completion with snake-venom phosphodiesterase (*Crotalus durissus*; Sigma) and summing the contributions from individual nucleotides. This value was adjusted to give the corrected value for the intact oligonucleotide, taking account of the hyperchromicity observed after digestion [9]. Final molar absorption coefficients, ϵ_{260}, for each oligonucleotide

Abbreviations used: DMS, dimethyl sulphate; g5p, gene 5 protein; G-quartet, guanine quartet; T_m, melting temperature.

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were 58445 (DNA-6), 135700 (DNA-16), 111050 (DNA-16AP), 148000 (DNA-16C) and 202365 (DNA-24) $M^{-1} \cdot cm^{-1}$. An ϵ_{260} of 147550 $M^{-1} \cdot cm^{-1}$ was used for RNA-16, as determined by O'Donohue [9].

Non-denaturing gel electrophoresis

End-labelled oligonucleotides were prepared in ST buffer [10 mM Tris/HCl (pH 8.0)/100 mM NaCl/1 mM EDTA] at a strand concentration of 1 μM. These were subsequently applied to a 10% (w/v) non-denaturing polyacrylamide gel (19:1 acrylamide-to-bisacrylamide ratio) containing 1 × TBE buffer (89 mM Tris/89 mM boric acid/2 mM EDTA) at 4 °C. The gel was vacuum-dried and bands were detected by exposure to a storage phosphor screen. Migration positions of the oligonucleotides were compared with an end-labelled oligonucleotide size marker (Pharmacia).

PhosphorImager analysis

A storage phosphor screen was exposed to vacuum-dried polyacrylamide gels. This allowed the detection and quantification of ^{32}P levels with a Molecular Dynamics PhosphorImager (and associated ImageQuant software).

High-resolution gel filtration

Oligonucleotides were applied to a Pharmacia Superose 12HR exclusion chromatography column equilibrated in ST buffer, running at a flow-rate of 0.4 ml/min. Elution profiles were monitored at 260 nm with a Jasco UV-975 detector interfaced to an IBM-PC compatible computer.

CD spectroscopy

CD spectra were collected on a Jasco J720 spectropolarimeter in a 0.2 cm pathlength cell. All CD data represent an average of three to five scans, baseline-corrected for signals due to the sample buffer and cell. The sample temperature was maintained by a thermostatically controlled water bath to an accuracy of 0.2 °C. Spectra were measured at strand concentrations of 18.4, 16.3, 18.8 and 7.3 μM for DNA-16(S), DNA-16(U), RNA-16(S) and DNA-6 respectively; the results were corrected to units of molar ellipticity. For the thermal denaturation experiments samples were heated at 1 °C/min and the temperature was monitored directly with a thermal probe.

Cation dependence

In a modified version of the experiment of Sen and Gilbert [10], samples of ^{32}P end-labelled DNA-16 (0.5 μM) were incubated for 16 h at 20 °C in 20 mM Tris/HCl (pH 8.0)/1 mM EDTA supplemented with 50 mM NaCl, KCl, LiCl, CsCl or RbCl. The samples were then applied to an 8% (w/v) non-denaturing polyacrylamide gel containing 1 × TBE buffer.

Dimethyl sulphate (DMS) protection and interference

Methylation protection reactions contained 2 μg/μl ^{32}P end-labelled DNA-24 in TE buffer [10 mM Tris/HCl (pH 8.0)/0.1 mM EDTA] with the addition of either NaCl or KCl to a final concentration of 100 mM. DMS was added to each reaction to a final concentration of 0.05% (v/v); stock DMS solution was diluted in 50 mM sodium cacodylate. Reaction volumes were typically 30 μl. Samples were then incubated at room temperature for 30 min before the addition of 2.5 vol. of ethanol to quench the methylation reaction. Samples were recovered by centrifugation, washed with 70% (v/v) ethanol, dried in vacuum and resuspended in TE buffer supplemented with 10 mM NaCl or 10 mM KCl. Samples were then applied to an 8% (w/v) non-denaturing preparative gel. Bands corresponding to form S (structured) and form U (unstructured) of the oligonucleotide were identified, then excised and eluted into the relevant TE/10 mM salt buffer. Purified form S and form U of the oligonucleotide were then treated with piperidine [11] and analysed on a 20% (w/v) denaturing (sequencing) gel.

Methylation interference reactions contained 2 μg/μl ^{32}P end-labelled DNA-24 in 50 mM sodium cacodylate containing 0.05% (v/v) DMS. Reactions were incubated at room temperature for 30 min, then quenched with ethanol as in the protection experiments. Samples were recovered by centrifugation, washed with 70% (v/v) ethanol and dried in vacuum before being resuspended in TE buffer supplemented by the inclusion of 100 mM NaCl or 100 mM KCl. Samples were then incubated at room temperature for a further 19–24 h before being applied to a preparative non-denaturing 8% (w/v) polyacrylamide gel. Eluted form S and form U of the oligonucleotide were treated with piperidine, then prepared and analysed as in the protection experiments.

RESULTS

Oligonucleotides d($GT_5G_4CT_4C$) and r($GU_5G_4CU_4C$) exist in two structural forms

When HPLC-purified oligonucleotide DNA-16 was run on a 10% (w/v) non-denaturing polyacrylamide gel, two distinct

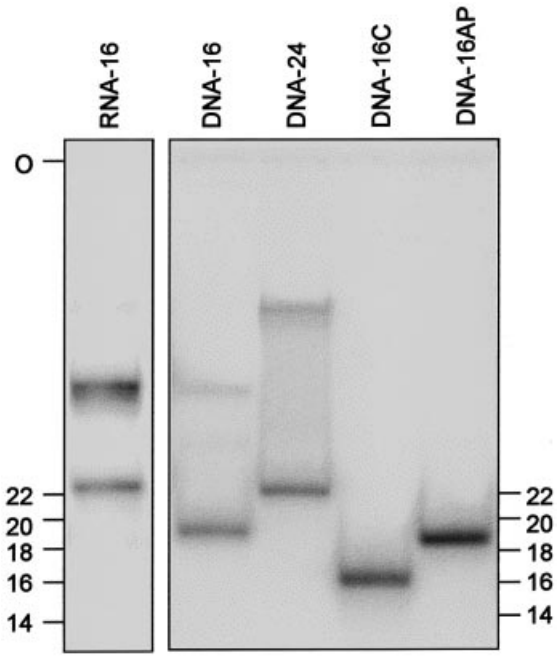

Figure 1 Electrophoretic pattern of ^{32}P end-labelled oligonucleotides

Samples were analysed by non-denaturing 10% (w/v) PAGE. Each lane contained oligonucleotide at a strand concentration of 1 μM in ST buffer. The origin of the gel is shown (O) together with the molecular sizes (in bases) of marker oligonucleotides.

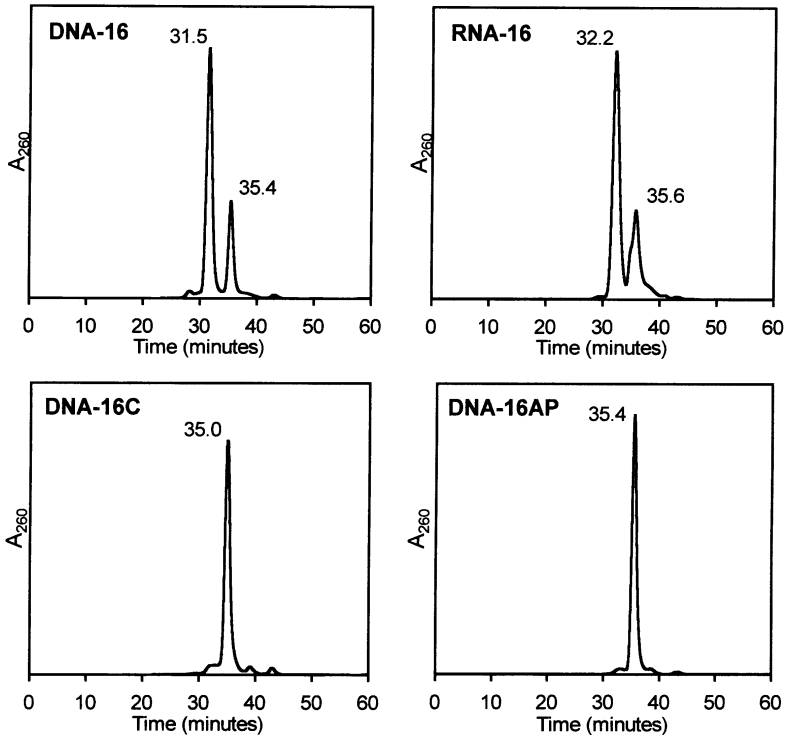

Figure 2 High-resolution gel-filtration profiles of oligonucleotides

Samples were applied to a Superose 12HR column at a strand concentration of 25 μM. The A_{260} of the column eluate was monitored. Retention times (in min) are shown for each peak detected. Structured and unstructured forms were eluted at approx. 32 and approx. 35 min respectively in each case.

bands were seen (Figure 1). In comparison with oligonucleotide size markers, it was the upper band that migrated anomalously, the lower band running with a mobility between that of 18-base and 20-base size markers. This suggests that the slowly migrating species corresponded to a structured form of the oligonucleotide (form S) and the faster species to the unstructured single-stranded form of the DNA (form U).

Samples of DNA-16 were also run on a gel-filtration column (Superose 12HR). Again, two species were seen for the oligonucleotide (Figure 2). The two species were isolated and run separately on non-denaturing gels. It was confirmed that the two elution peaks corresponded to the two bands previously seen by gel electrophoresis, the higher-molecular-mass peak observed by gel filtration corresponding to the lower-mobility band on the gel (results not shown). When the isolated species were run on denaturing (sequencing) gels, they had identical mobilities, confirming that the two species were different structural forms rather than impurities or degradation products.

When both DNA species were purified by gel-filtration chromatography and re-applied to the column, form U was eluted as a single peak at its original retention time. Form S, however, also gave rise to a major peak (at its characteristic retention time) but, in addition, a smaller peak corresponding to form U was generally observed. Subsequent experiments showed that the tendency of the structured species to revert, in part, to the unstructured form was dependent on DNA concentration and on the time and temperature at which the sample was stored after the initial separation procedure. In particular, the stability of the structured species was favoured by high concentration and by low temperature. These results suggest that the two forms are in very slow equilibrium, the point of equilibrium depending on concentration, as expected for an intermolecular strand association.

The equivalent RNA sequence r($GU_5G_4CU_4C$) (RNA-16) was also investigated by non-denaturing gel electrophoresis and by high-resolution gel filtration (Figures 1 and 2). Again, two species were observed, indicating that the RNA sequence is capable of adopting the structured conformation.

Additional experiments were performed with the oligonucleotide sequence DNA-16C, corresponding to the reverse complement of DNA-16 (Figure 1 and 2). By both non-denaturing PAGE and by gel filtration, only a single species was observed, the mobility of which was close to that of the unstructured form of DNA-16 (the small difference in electrophoretic mobility is likely to have reflected differences in base composition). To examine the effect of more subtle differences, the oligonucleotide DNA-16AP was synthesized in which one of the guanine residues (G-8) was replaced by 2-aminopurine (removing both the O6 and the N1 imino proton from the guanine base). The results show that replacement of just one guanine residue with 2-aminopurine is sufficient to inhibit structure formation (Figures 1 and 2).

To look at the effect of sequence context, a longer, 24-base, DNA sequence was examined. The oligonucleotide sequence of DNA-24 included the sequence found in DNA-16 but with an extra four bases (taken from the genomic sequence of the bacteriophage) at both the 5′ and 3′ ends of the oligonucleotide. As observed for the shorter 16-base sequence, DNA-24 also exhibited two structural forms on electrophoresis (see Figure 1) and gel filtration (results not shown). This sequence was used for subsequent DNA-methylation experiments.

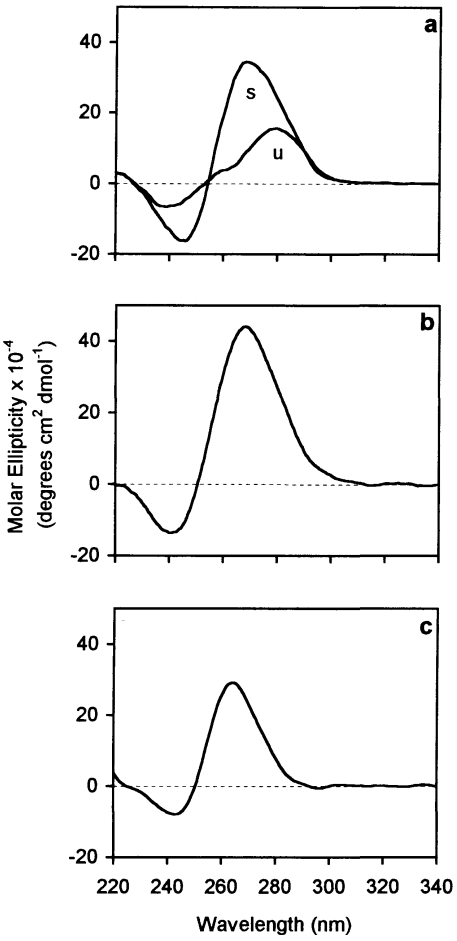

Figure 3 CD spectra of oligonucleotides

(**a**) DNA-16; (**b**) RNA-16; (**c**) TG_4T. In (**a**) the spectra of the structured (S) and unstructured (U) forms are shown for comparison. Spectra were measured at 4 °C in ST buffer. CD is shown in units of molar ellipticity.

Dependence of structure formation on univalent cations

For a number of structures containing non-Watson–Crick base-pairs, it has been shown that the stability and formation of structure is dependent on the cations present in solution. We therefore investigated the degree of structure formation in DNA-16 with a range of univalent cations (Li, Na, K, Cs and Rb, each at 50 mM). We found that structure formation was dependent on the cation. Under the conditions tested, K^+, Na^+ and Rb^+ were all effective in promoting structure formation; in contrast, little or none of the structured form was observed in the presence of Cs^+ or Li^+ (results not shown).

CD spectroscopy of DNA-16 and RNA-16

The two forms of DNA-16 were separated and purified as before by gel-filtration chromatography. The CD spectra of each form [designated DNA-16(U) and DNA-16(S)] were then measured (Figure 3a). The CD spectrum of form U was similar to that of other single-stranded oligonucleotides, e.g. $d(AC)_{12}$, with a positive peak at 275–280 nm and a negative peak of comparable intensity at approx. 240 nm [12]. In contrast, the CD spectrum of form S was significantly asymmetric, with a large positive peak at 268 nm and a smaller negative peak at 246 nm. This spectrum resembled the CD spectra of parallel-stranded guanine tetraplexes, such as those formed with the sequences dT_4G_4 [13,14], dT_4G_4T [14] and G_4TG_4 [15]. CD spectra were also recorded of RNA-16 (Figure 3b), again after isolation of the structured form [RNA-16(S)] by gel filtration. The CD spectrum of RNA-16(S) closely resembled that of DNA-16(S), although the positive peak at 268 nm was somewhat sharper and of greater intensity.

The hexanucleotide $d(TG_4T)$ forms a very stable tetraplex structure that has been determined at high resolution by both X-ray crystallography [16] and NMR [17]. For comparison, we measured the CD spectrum of this sequence (DNA-6). Again, the oligonucleotide was purified by gel filtration, although in this case almost none of the unstructured form was observed. The CD spectrum of this sequence (Figure 3c) was typical of other G-quartet structures and served as a reference spectrum.

Stabilization of structure by K^+ ions

By following reported procedures [10], the temperature dependence of structure formation was initially investigated by heating samples of DNA-16 to temperatures in the range 35–75 °C, followed by snap-cooling on ice and the immediate application to a non-denaturing polyacrylamide gel. The results showed clearly that the structured form was favoured at low temperature and suggested that, under the conditions used [20 mM Tris/HCl (pH 8.0)/100 mM NaCl/1 mM EDTA] the structure could no longer be formed at temperatures above 55 °C (results not shown).

Thermal denaturation experiments were also performed by monitoring the UV absorption of the DNA at 260 nm, but no clear transition was observable by this technique. However, subsequent investigation of the CD spectra of DNA-16(S) as a function of temperature showed a very clear transition from the structured to the unstructured form in buffers containing 100 mM NaCl (Figure 4). There was a marked change in the form of the CD spectrum of the structured form as the temperature was increased, undergoing a transition with a melting temperature (T_m) of approx. 47 °C. At temperatures of 30 °C or below, there was little change in the form of the CD spectrum, whereas at 60 °C or above, the spectrum resembled that of form U. In contrast, in experiments with buffers containing 100 mM KCl, the structured form persisted at all temperatures measured.

Parallel CD melting experiments were performed for RNA-16(S). In 100 mM NaCl, the RNA structure was clearly more stable than the equivalent DNA structure, having a T_m of approx. 62 °C (Figure 4). The RNA structure was also stabilized by K^+ ions and, as observed for the DNA sequence, there was no transition to the unstructured form at all temperatures measured. However, there was a gradual decrease in the CD signal with increasing temperature (a similar trend could be seen in the presence of Na^+ ions). The higher T_m of the RNA sequence, and the greater magnitude of the CD signal (34×10^4 compared with 40×10^4 degrees·cm²·dmol^{-1} at 10 °C) suggests that there was some degree of additional structure in the RNA. However, this additional structure must have been relatively weak and not stabilized specifically by K^+ because the magnitudes of the CD signals at high temperatures in K^+ were comparable for DNA and RNA.

DMS protection and interference

DMS protection and interference assays [11] have been widely used by a number of workers [10,18–20] as a probe for the presence of G-quartets, because N7 in each guanine in a G-quartet is involved in hydrogen-bonding to the N2H of another

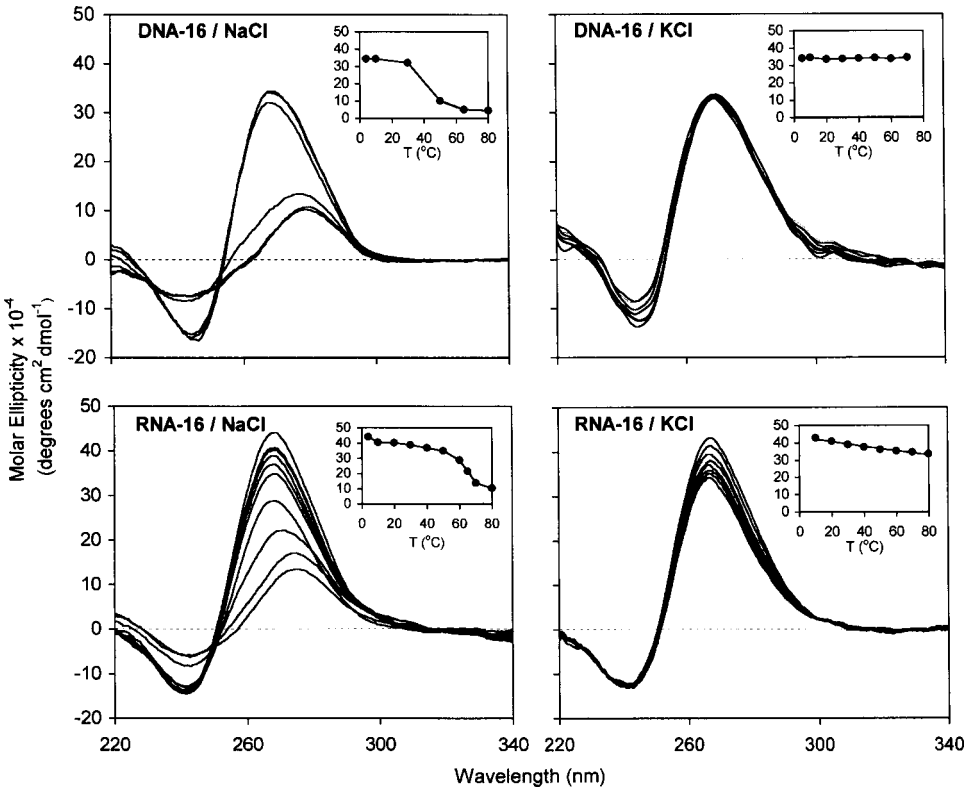

Figure 4 CD-monitored thermal denaturation profiles of oligonucleotides

The CD spectra for oligonucleotides DNA-16(S) and RNA-16(S) were measured over a range of temperatures (in buffers containing Na^+ or K^+). The molar ellipticity values at 268 nm as a function of temperature are shown in each inset.

guanine base. To improve resolution, and to include bases outside the 16-base sequence as an internal control on the gel, the oligonucleotide DNA-24 was used as a substrate for DMS protection and interference assays.

Methylation interference assays (Figure 5a) indicate that methylation of any of the central guanine residues (G-11–G-14 in DNA-24, corresponding to G-7–G-10 in DNA-16) is sufficient to prevent structure formation. This suggests that the N7 nitrogens of all four central guanine bases were involved in hydrogen-bonding. However, the results of the methylation protection experiments (Figure 5b) indicate that only the two central guanine residues (G-12 and G-13 in DNA-24, corresponding to G-8 and G-9 in DNA-16) were fully protected from methylation, with little or no protection for the flanking bases G-10 and G-14. The guanine residues at each end of DNA-24 (G-5 and G-22) seem to have been accessible to modification; however, there is some evidence that methylation of G-5 impaired the ability of the DNA to adopt the structured form (Figure 5a) and it is possible that this, too, was involved in G-quartet formation. For G-22, there was no effect of methylation in either protection or interference experiments, as expected because it was outside the 16-base sequence corresponding to DNA-16.

DISCUSSION

We have shown that the oligonucleotide d($GT_5G_4CT_4C$) can adopt two distinct structural forms that are reasonably stable and can be separated by chromatographic procedures, although their exact proportions vary with the prevailing ionic conditions. A variety of experiments have shown that one form (form U) co-migrates with other oligonucleotides of the same size but different sequence, and lacks secondary structure. However, the second species (form S) has a quite different mobility on non-denaturing gels, has a much higher apparent molecular mass by gel filtration and has considerable secondary structure.

The blocks of guanine and thymine bases in the sequence suggest that the structure might be related to the tetraplex structures formed by sequences such as d(TG_4T), d(G_4TG_4) and similar oligonucleotide structures based on telomeric DNA sequences [21]. These sequences can self-associate to form a four-stranded structure linked through the formation of G-quartets involving Hoogsteen-type hydrogen bonds [10].

We have used a number of techniques to probe the structure formed in DNA-16, RNA-16 and related oligonucleotides. CD spectroscopy confirms that form S is highly structured. The CD spectrum (for the structured form of both DNA-16 and RNA-16) is similar to that for parallel-stranded guanine tetraplexes. Parallel tetraplexes have a largely asymmetric CD spectrum, with a positive peak centred at approx. 264 nm, and a smaller negative peak at approx. 242 nm [13–15,22]. In contrast, antiparallel tetraplexes have distinctly different CD spectra, with a major positive peak at 295 nm and a minor negative peak at approx. 265 nm [14,16,23–25]. The CD signal from RNA-16(S) is somewhat larger than that from its DNA equivalent; this could indicate that there is some structure outside of the central core, for example the formation of uracil quartets, which have been

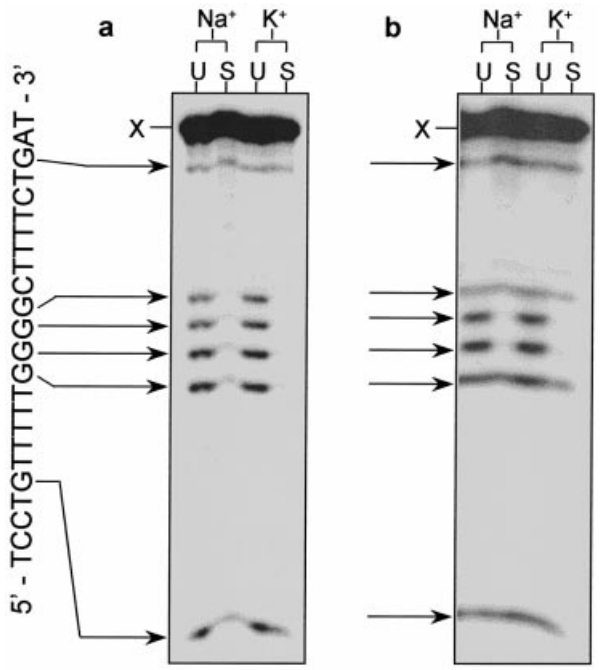

Figure 5 DMS footprinting

(**a**) DMS interference assay. Oligonucleotide DNA-24 was chemically modified with DMS in the absence of univalent cations. Na^+ or K^+ ions were subsequently added to promote structure formation. Each oligonucleotide form (form U and form S, lanes marked U and S respectively) was then isolated by preparative gel electrophoresis. Sites of modification were subsequently detected by denaturing PAGE analysis after depurination with piperidine and strand cleavage. The sequence of DNA-24 is shown for reference. X denotes full-length non-modified oligonucleotide. (**b**) DMS protection assay. Oligonucleotide DNA-24 was chemically modified with DMS in the presence of either 100 mM Na^+ ions or 100 mM K^+ ions ,as indicated. Each oligonucleotide form was then isolated, treated with piperidine and detected as for (**a**).

reported in at least one example of an RNA tetraplex [25]. The enhanced stability of the RNA sequence to thermal denaturation (T_m approx. 62 °C compared with approx. 47 °C for the DNA sequence) also indicates that additional structure is present in the RNA.

Tetramerization of sequences containing guanine bases is dependent on the presence of univalent cations [10,19], with either K^+ or Na^+ ions promoting the greatest formation of structure in solution. We have shown that the structure formed by DNA-16 is similarly cation-dependent, with K^+, Na^+ or Rb^+ (but not Cs^+ or Li^+) ions promoting structure formation, as is commonly found for other tetraplexes [10,19,22,26,27]. The structured forms of DNA-16 and RNA-16 are markedly resistant to thermal denaturation in the presence of K^+ ions. A similar stabilization of G-quartet structure has been observed for other sequences in the presence of K^+ [10].

Methylation protection experiments show that two of the four central guanine residues are fully protected from methylation at the N7 position, the other two guanine residues in this 'core region' are less protected (in both NaCl and KCl containing buffers). Interference studies, in contrast, show that methylation of any of the four guanine bases interferes with structure formation. Whereas methylation interference experiments probe the involvement of N7 of guanine in hydrogen-bonding interactions, methylation protection experiments are a measure of the accessibility of N7 to attack by DMS. Therefore it might be expected that N7 of the outer bases of the proposed tetraplex region might be more accessible to the reagent than those of the inner guanine residues, as observed.

Under the conditions tested, substitution of 2-aminopurine for guanine G-8 in DNA-16 effectively prevents structure formation (as judged by non-denaturing gel electrophoresis and gel filtration). This substitution removes two functional groups, the O6 and the N1 (imino) proton, from the guanine base; both of these are closely involved in the cyclic hydrogen-bonding pattern seen in G-quartets [18–20,28]. The O6 group is additionally involved in the co-ordination of univalent cations either between or within the planes of each quartet [16]; its removal would therefore be particularly destabilizing.

An anomalously migrating species for the sequence DNA-16 was first observed by Michel and Zinder [7] in gel retardation experiments with g5p of bacteriophage fd. They proposed that the slower-migrating species might arise from the annealing of two oligonucleotide strands through the formation of G–T base-pairs. Our experiments, however, present strong evidence that the interaction involves the formation of G-quartets. Surprisingly, only one band was detected for RNA-16 in the earlier experiments [7]. We have shown that the two structural forms are also seen for the RNA sequence. The apparent absence of form-S RNA in the earlier experiments [7] could be explained by the low concentration of oligonucleotide used (0.2 μM, 1/50 of the DNA concentration) because the phenomenon is concentration dependent.

The existence of proteins that recognize telomeric DNA sequences suggests that G-quartet structures do have an important biological function [21]. Similarly, preliminary evidence [29] suggests that g5p binds preferentially to the structured form of the 16-base sequence d($GT_5G_4CT_4C$), forming a well-defined complex that is quite distinct from the complex with the unstructured form (A. W. Oliver, E. Schroeder and G. G. Kneale, unpublished work); this might be related to the role of g5p in the replication of the single-stranded viral genome. Biological roles for G-quartet structures in RNA are less well documented. The known preference of g5p for binding to the RNA sequence r($GU_5G_4CU_4C$) [7] could be a consequence of its ability to form tetraplex or related structures; if so, this would be the first example of a role for G-quartets in translational control. Detailed studies on the interaction of fd g5p with the structured and unstructured forms of DNA-16 and RNA-16 are currently in progress to elucidate the nature of these interactions.

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REFERENCES

1 Kneale, G. G. (1992) Curr. Opin. Struct. Biol. **2**, 124–130
2 Model, P. and Russel, M. (1988) The Bacteriophages, vol. 2., Plenum, New York
3 Gray, C. W. (1989) J. Mol. Biol. **208**, 57–64
4 Model, P., McGill, C., Mazur, B. and Fulford, W. D. (1982) Cell **29**, 329–335
5 Yen, T. S. and Webster, R. E. (1982) Cell **29**, 337–345
6 Michel, B. and Zinder, N. D. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 4002–4006
7 Michel, B. and Zinder, N. D. (1989) Nucleic Acids Res. **17**, 7333–7344
8 Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
9 O'Donohue, M. J. (1991) Ph.D. Thesis, School of Biological Sciences, University of Portsmouth
10 Sen, D. and Gilbert, W. (1990) Nature (London) **344**, 410–414
11 Maxam, A. M. and Gilbert, W. (1980) Methods Enzymol. **65**, 499–500
12 Clark, C. L., Cecil, P. K., Singh, D. and Gray, D. M. (1997) Nucleic Acids Res. **25**, 4098–4105
13 Guo, Q., Lu, M., Marky, L. A. and Kallenbach, N. R. (1992) Biochemistry **31**, 2451–2455

14 Lu, M., Guo, Q. and Kallenbach, N. R. (1992) Biochemistry **31**, 2455–2459
15 Balagurumoorthy, P., Brahmachari, S. K., Mohanty, D., Bansal, M. and Sasisekharan, V. (1992) Nucleic Acids Res. **20**, 4061–4067
16 Laughlan, G., Murchie, A. I. H., Norman, D. G., Moore, M. H., Moody, P. C. E., Lilley, D. M. J. and Luisi, B. (1994) Science **265**, 520–524
17 Abdoul-ela, F., Murchie, A. I. H., Norman, D. G. and Lilley, D. M. J. (1994) J. Mol. Biol. **243**, 458–471
18 Sen, D. and Gilbert, W. (1988) Nature (London) **334**, 364–366
19 Williamson, J. R., Raghuraman, M. K. and Cech, T. R. (1989) Cell **59**, 871–880
20 Sundquist, W. I. and Klug, A. (1989) Nature (London) **342**, 825–829
21 Rhodes, D. and Giraldo, R. (1995) Curr. Opin. Struct. Biol. **5**, 311–322
22 Hardin, C. C., Henderson, E., Watson, T. and Prosser, J. K. (1991) Biochemistry **30**, 4460–4472
23 Jin, R., Breslauer, K. J., Jones, R. A. and Gaffney, B. L. (1990) Science **250**, 543–546
24 Williamson, J. R. (1993) Curr. Opin. Struct. Biol. **3**, 357–362
25 Cheong, C. and Moore, P. (1992) Biochemistry **31**, 8406–8414
26 Kim, J., Cheong, C. and Moore, B. (1991) Nature (London) **351**, 331–332
27 Venczel, E. A. and Sen, D. (1993) Nucleic Acids Res. **32**, 6220–6228
28 Gellert, M., Lipsett, M. N. and Davies, D. R. (1962) Proc. Natl. Acad. Sci. U.S.A. **48**, 2013–2018
29 Oliver, A. W. and Kneale, G. G. (1997) Biochem. Soc. Trans. **25**, S643

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