Circular Dichroism of DNA Frayed Wires

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ABSTRACT Ultraviolet circular dichroism spectra are reported for the oligonucleotide $d(A_{15}G_{15})$ in aqueous solutions containing 5 mM $MgCl_2$ at several temperatures and in the presence of partially complementary oligonucleotides. Oligonucleotides with several consecutive terminal guanine residues self-associate to form aggregates, called frayed wires, that consist of integer numbers of strands. A "stem" is formed through interactions between the guanine residues of the associated oligonucleotides, whereas the adenine "arms" remain single stranded. Upon subtracting the circular dichroism spectrum of $d(A_{15})$ from that of $d(A_{15}G_{15})$, one obtains a spectrum that closely resembles previously published spectra of poly(G). Subtracting spectra measured at temperatures between 10°C and 60°C reveals the resultant spectra to be independent of temperature, consistent with the extreme thermal stability observed for the aggregated structures. Upon the addition of $d(T_{15})$ to the solution, complexes with the adenine portion of the $d(A_{15}G_{15})$ frayed wires are formed. Subtraction of $d(A_{15}):d(T_{15})$ spectra measured at several temperatures from those of the $d(A_{15}G_{15}):d(T_{15})$ does not significantly alter the spectrum of the guanines. The helix-coil transition temperature of $d(A_{15}):d(T_{15})$ duplex is identical to that of the unbinding of $d(T_{15})$ from $d(A_{15}G_{15}):d(T_{15})$ complexes. Experiments using oligonucleotides in which the adenines were replaced with sequences of bases yielded similar results. By varying the length of the nonguanine tract, it is shown that the solubility of the complexes increases with the length of the nonguanine region of the oligonucleotide.

INTRODUCTION

DNA frayed wires are extremely stable multimolecular species that arise from the spontaneous aggregation of oligonucleotides containing several consecutive terminal guanine residues (Protozanova and Macgregor, 1996). For example, $d(A_{15}G_{15})$ self-associates to form high-molecular-weight complexes that resolve as a regular ladder of bands during electrophoresis on denaturing and nondenaturing polyacrylamide gels. The ability to resolve these structures, even under conditions that denature standard DNA complexes, attests to their stability. Using gel electrophoresis, we demonstrated that the high-molecular-weight aggregates interact with partially complementary oligonucleotides, such as dT_{10}, but not with dC_{10}. On the basis of this result we proposed that the adenines in the $d(A_{15}G_{15})$ frayed wires are available for A:T duplex formation. The inability of the guanine residues to interact with dC_{10} implies that they are already involved in an interaction. Thus the aggregates comprise two structural domains: single-stranded adenine runs or "arms," and self-complexed guanines or the "stem." In our original publication, we reported that the binding of dT_{10} to the arms of frayed wire did not alter the distribution of individual strands between different species. This observation suggested that the interaction in the arms does not affect the stem of the complex, and thus the two structural domains are independent.

In this work we have used circular dichroism spectroscopy to study the structural and thermodynamic properties of the arm and stem regions of frayed wires formed by $d(A_{15}G_{15})$. Circular dichroism (CD) spectroscopy is widely used to characterize the conformation of biological molecules. The differential absorption of right- and left-hand polarized light arises from the chirality of the chromophore. For DNA, the individual nucleotides and their environments are asymmetrical, and the CD signal provides a convenient way to assess changes in the conformational state of oligonucleotides and polymers as a function of temperature, pressure, ionic strength, and other parameters. Because of the dependence of the CD signal on the symmetry of the molecular environment of the chromophore, DNA exhibits CD signals that are characteristic of various conformational states. The A-, B-, and Z-conformations of DNA exhibit CD spectra that are sufficiently distinct that it is often possible to directly monitor the presence of a particular conformation. This technique is also useful in the investigation of unusual multiple-stranded DNA structures (Edwards et al., 1990; Marotta et al., 1996; Chen, 1995).

Our previous data have shown that the guanine residues of the stem are involved in mutual interactions that we have not yet characterized. One nonstandard DNA conformation that is possibly related to the guanine-guanine interactions in frayed wires and has been studied by CD spectroscopy is the guanine tetraplex, a self-complexed structure composed of DNA containing runs of consecutive guanines (Williamson, 1994). The CD spectrum of poly(dG), reported by Gray and Bollum in 1974, as well as the recent spectra of a number of telomere-like oligonucleotides, e.g., $d(TG_3T)$ and $d(T_4G_4)$, display a strong maximum in the vicinity of 257–262 nm and a trough at ~240 nm (Gray and Bollum, 1974; Jin et al., 1992; Lu et al., 1992). On the basis of the

Received for publication 18 March 1998 and in final form 8 May 1998.

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0006-3495/98/08/982/08 $2.00

guanine tetrad structures observed in crystallographic and NMR studies of d(TG_4T), this spectrum was assigned to the tetraplex with a parallel orientation of strands (Laughlan et al., 1994; Aboul-ela et al., 1994). However, under defined conditions, telomere-like oligonucleotides containing two or more runs of guanine residues can exhibit CD spectra with a positive band at ~295 nm and a negative band at ~262 nm (Lu et al., 1993; Balagurumoorthy et al., 1992). Such spectra arise from a foldback structure with antiparallel strand alignment (Kang et al., 1992; Smith and Feigon, 1993).

In this study we show that the CD spectra of d($A_{15}G_{15}$) frayed wires formed in aqueous buffer containing 5 mM $MgCl_2$ can be accurately described by the simple addition of the spectra of the two contributing structural components, i.e., dA_{15} + dG_{15}. The spectra of the stem region are similar, although not identical, to the spectra of poly(dG). Neither the interaction of a complementary strand with the arms nor changes in temperature alter the spectrum of the stem. The thermal stability of the duplexes formed by the interaction of a complementary oligonucleotide with the arms is identical to the stability of the isolated duplex. Finally, we show that the arms play an important role in keeping the high-molecular-weight aggregates in solution.

EXPERIMENTAL

DNA

We purchased the cartridge-purified synthetic oligonucleotides used in our studies from the Hospital for Sick Children/Pharmacia Biotech Centre at the University of Toronto. Their concentrations were estimated spectrophotomerically, using extinction coefficients at 260 nm calculated from the nearest-neighbor model (Cantor and Tinoco, 1965; Cantor et al., 1979). Lyophilized DNA was resuspended in an appropriate volume of Baker ultrapure water (Baker Chemical) to give 100 μM (strands) stock solutions and stored at −20°C. Samples of frayed wires were prepared by diluting an aliquot of the stock solution in 90 mM Tris borate (pH 8.3), 5 mM $MgCl_2$ (TBM), or 10 mM Tris-HCl (pH 8.0), 1 M NaCl, such that the DNA concentration was between 2.5 and 5.0 μM (strands). After the solution was heated to 100°C, it was allowed to cool slowly to room temperature, followed by incubation at 10°C for 10–15 h. The samples of the short oligonucleotides, dT_{15} and dA_{15}, as well as the duplex dA_{15}:dT_{15}, had final concentrations of 2.5 μM in single strands or duplexes.

Circular dichroism

CD spectra were recorded with an Aviv 62A DS circular dichroism spectropolarimeter (Aviv Associates, Lakewood, NJ). The spectral contributions of the cuvette and buffer were subtracted when necessary. The absorption spectrum for each sample was obtained using the CD instrument according to the protocol outlined in the manual.

To study the temperature dependence of the spectra, the desired temperature was set manually and allowed to equilibrate for 15 min before the measurement. Two spectra were collected at each temperature to ensure that the sample had reached equilibrium. CD melting curves were recorded by following the decrease in ellipticity at 246 nm with a heating rate of 0.6°C/min.

Centrifugation assay

Sedimentation analysis was performed in a microcentrifuge. Approximately 0.7 OD_{260} units of the oligonucleotide in 1 ml of 10 mM Tris-HCl, pH 8.0, 5 or 20 mM $MgCl_2$, was subjected to repetitive centrifugation at 12,000 or 10,000 × g for 5–20 min. After each centrifugation step, the supernatant was placed in an optical cell, and the absorbance spectrum was collected with a Uvikon model 860 spectrophotometer (Kontron). The results are reported in terms of the ratio of the change in the optical density at the 252 nm maximum relative to the initial OD reading at 252 nm (recorded upon the addition of $MgCl_2$).

Nondenaturing gel electrophoresis experiments

Radiolabeling was carried out by reacting 10 pmol of oligonucleotide, either d($A_{15}G_{15}$) or dT_{15}, and [γ-^{32}P]ATP (Amersham) in the presence of T4 polynucleotide kinase (Pharmacia). After mixing of d($A_{15}G_{15}$), and/or dT_{15}, and 2 μl of radioactively labeled oligonucleotide, the magnesium-containing buffer solution (TBM) was added. The samples were heated to 95°C, followed by slow cooling and incubation at 10°C for 24 h. The samples were 2 μM in strands of unlabeled d($A_{15}G_{15}$) and dT_{15}. Frayed wire ladders were resolved on native 10% polyacrylamide gels run at 3 V/cm at 10°C. The digitized image of the gel pattern was collected with an AMBIS radioanalytic imaging system (Bellerica, MA).

RESULTS

The stem of the polymer

Gel electrophoresis experiments have shown that oligonucleotides such as d($A_{15}G_{15}$), d($T_{15}G_{15}$), d($T_{12}G_{12}$), etc., self-assemble into extremely stable, high-molecular-weight species in the presence of millimolar concentrations of $MgCl_2$ (Protozanova and Macgregor, 1996; Poon and Macgregor, 1998). The behavior of the aggregated forms of d($A_{15}G_{15}$) during nondenaturing polyacrylamide gel electrophoresis is shown in Fig. 1, lane 1. Formation of the aggregates involves interactions between the 3′-terminal guanine residues. The adenines at the 5′ end of the oligo-

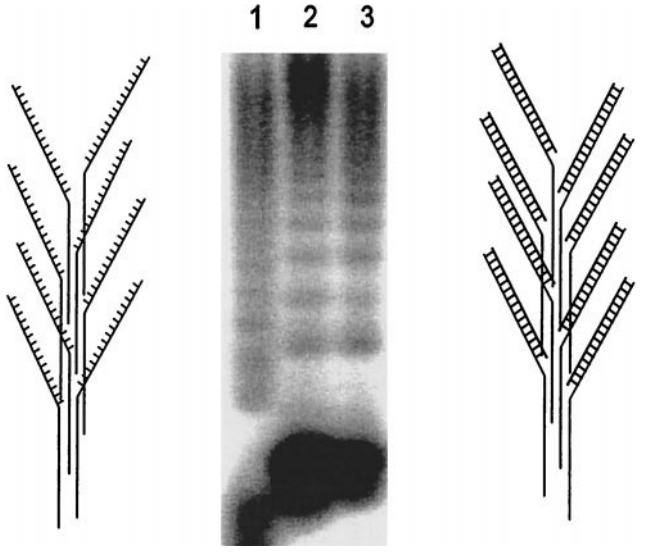

FIGURE 1 Electrophoresis of d($A_{15}G_{15}$) frayed wires in a native polyacrylamide gel. Lane 1, the band pattern of ^{32}P-labeled d($A_{15}G_{15}$) frayed wires alone. Lane 2, the band pattern of ^{32}P-labeled d($A_{15}G_{15}$) after the addition of dT_{15}, the oligonucleotide complementary to the arms. Lane 3, same oligonucleotides as in Lane 2, however, dT_{15} is radiolabeled. A schematic of the structure proposed for frayed wires is also shown.

nucleotide remain single stranded and can participate in standard base-pairing interactions with the complementary oligonucleotide, dT_{15} (*lanes 2 and 3*). Because of the proposed disposition of the two structural domains of these aggregates, and in deference to the G-wire structures formed by $d(G_4T_2G_4)$ (Marsh and Henderson, 1994), we have called these complexes *frayed wires*. To distinguish the two structural regions of frayed wires, we refer to the interacting guanines as the *stem* and the single-stranded region as the *arms*.

Circular dichroism spectra of $d(A_{15}G_{15})$ frayed wires formed in the presence of 5 mM $MgCl_2$ at temperatures ranging from 10 to 60°C are shown in Fig. 2 *A I*. The spectra exhibit a strong positive maximum at 262 nm, with a shoulder at ~275 nm and a negative maximum at 244 nm. These spectral features are conserved throughout the temperature range studied. Some minor changes occur in the absorption spectra upon heating to 60°C (Fig. 2 *A II*). The hyperchromicity at the 257-nm maximum at higher temperatures may arise from disruption of the base stacking of the 15 adenine residues at the 5′ end of the oligonucleotide. The absorption spectrum of the single-stranded oligonucleotide dA_{15} behaves in a similar manner, exhibiting hyperchromicity upon heating from 10°C to 60°C (Fig. 2 *B II*).

In our original paper, we presented qualitative data from electrophoresis gels showing that the arms of frayed wires can interact with complementary DNA oligonucleotides with no apparent change in the stability of the G-rich stem (Protozanova and Macgregor, 1996). This result suggested that the two sequence regions of the oligonucleotide behave as independent domains. Based on this idea and our working model of the structure of frayed wires, we hypothesized that the CD spectrum of a frayed wire would be approximated by the sum of the CD spectra of a stem region, i.e., poly(dG), and an arm region, single-stranded dA_{15}. In Fig. 2, *C I* and *II*, we present the CD and OD difference spectra obtained by subtracting the spectrum of dA_{15} (Fig. 2, *B I* and *II*) from that of $d(A_{15}G_{15})$. The CD difference spectrum is similar to that of the self-complexed form of poly(dG), with a positive maximum at 257 nm, a trough at 240 nm, and almost zero ellipticity at wavelengths longer than 280 nm (Gray and Bollum, 1974). The absorbance difference spectrum has a characteristic shoulder at 280 nm and resembles the spectrum of poly(dG). Whereas the absorbance spectrum does not change with increasing temperature, there is a small decrease in ellipticity of the 257-nm band in the CD spectrum.

Interaction of the arms with complementary oligonucleotides

We have investigated whether the interaction of the oligonucleotide complementary to the arms, dT_{15}, will influence the spectrum of the stem. The CD spectrum changes significantly upon the addition of dT_{15} to the $d(A_{15}G_{15})$ sample (Fig. 2 *A I*). To assess the effect of double-stranded arms on the properties of the stem, we collected the spectrum of the double-stranded region, the CD spectrum of the standard duplex dA_{15}: dT_{15} is presented in Fig. 2 *B I*. Comparison of the spectra in Fig. 2 *A I* with those in Fig. 2 *B I* shows that dA_{15} and $d(A_{15}G_{15})$ undergo similar spectral changes upon interaction with dT_{15}. A shoulder appears at 280 nm and the amplitude of the peaks increases, especially the minimum at 245 nm, which becomes ~2.5 times more intense. Changes

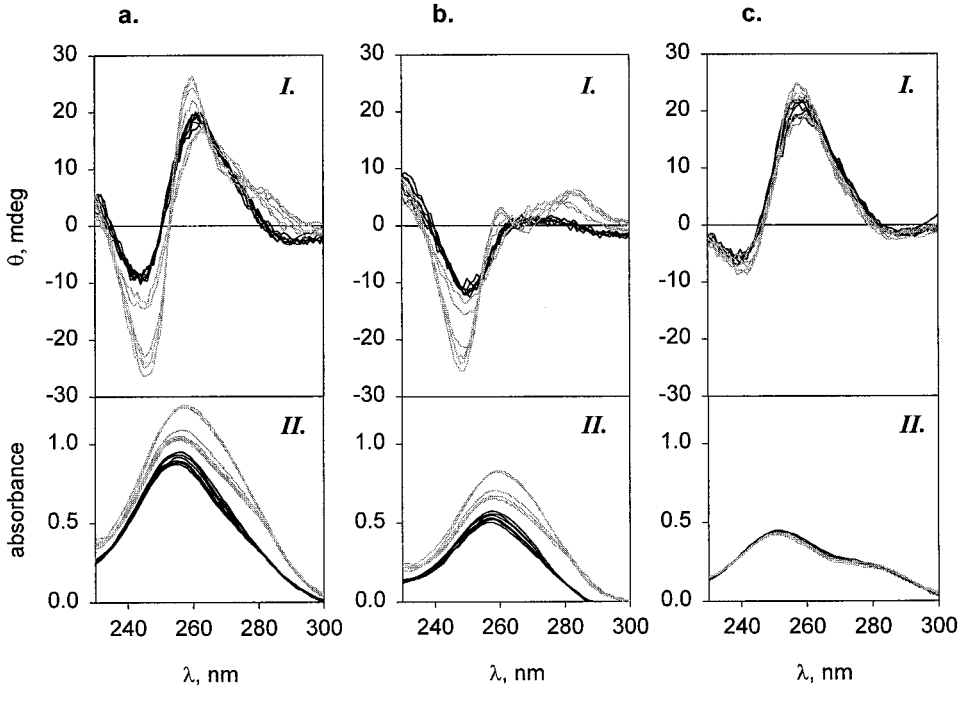

FIGURE 2 Temperature dependence of CD (panel *I*) and OD (panel *II*) spectra of (*a*) frayed wires arising from $d(A_{15}G_{15})$ (*black*) and the complex $d(A_{15}G_{15})$:dT_{15} (*gray*); (*b*) dA_{15} (*black*) and dA_{15}:dT_{15} (*gray*); (*c*) difference spectra representing the guanine stem of frayed wire calculated as $d(A_{15}G_{15})$ − dA_{15} (black) and $d(A_{15}G_{15})$:dT_{15} − dA_{15}:dT_{15} (*gray*). Spectra were recorded every 10°C from 10°C to 60°C. Samples were prepared in TBM buffer at 5 μM (strands) $d(A_{15}G_{15})$ or dA_{15} and 5 μM dT_{15}, where applicable.

in the absorbance spectra reflect the increase in the overall DNA content upon the addition of dT_{15} (Fig. 2 *A II*) and are comparable to the changes in the dA_{15} series after the addition of dT_{15} (Fig. 2 *B II*).

Fig. 2, *C I* and *II*, exhibits the CD and OD difference spectra, respectively, resulting from subtraction of the spectra of the dA_{15}:dT_{15} duplex from those of the $d(A_{15}G_{15})$:dT_{15}. The CD and OD difference spectra are nearly identical to the spectra of the stem of uncomplexed frayed wires shown in the same panels of these figures. The similarity between the difference spectra obtained from the complexed (upon addition of dT_{15}) and the free forms of frayed wires indicates the structural independence of the stem and the arms. The presence of the stem does not significantly influence the conformation of the arms, or vice versa.

The influence of temperature on the spectra

As a solution containing $d(A_{15}G_{15})$:dT_{15} is heated from 10°C to 60°C, the CD spectrum changes in shape and amplitude (Fig. 2 *A I*). The sharp positive and negative maxima disappear, and at 60°C the spectrum is similar to that of $d(A_{15}G_{15})$. As shown in Fig. 2 *C I*, the difference spectrum at each temperature is the same as the stem spectrum of $d(A_{15}G_{15})$ alone. We conclude that the temperature-induced spectral changes recorded in Fig. 2 *A I* arise from separation of the strands of the dA_{15}:dT_{15} duplex. Fig. 3 demonstrates the absence of interaction between $d(A_{15}G_{15})$ frayed wires and dT_{15} at 60°C. The spectrum of the mixture $d(A_{15}G_{15})$ + dT_{15} is the same as the sum of the spectra of $d(A_{15}G_{15})$ and dT_{15} measured separately.

Closer inspection of the temperature dependence of the spectra of the frayed wire:dT_{15} complex (Fig. 2 *A I*) reveals that the majority of the change occurs between 40°C and 50°C. We measured the temperature dependence of the CD signal at 246 nm for the $d(A_{15}G_{15})$:dT_{15} complex and the dA_{15}:dT_{15} duplex under the same experimental conditions. As shown in Fig. 4, the intensity of the 246-nm band is superimposable for the two samples throughout the temperature range. Both $d(A_{15}G_{15})$:dT_{15} and dA_{15}:dT_{15} exhibit a cooperative transition with a midpoint temperature of ~47°C. Based on these results, we infer that the presence of the stem in the $d(A_{15}G_{15})$:dT_{15} complex does not significantly affect the stability of the duplex formed between the arm of frayed wire and a complementary strand.

The difference spectra in Fig. 2 *C I* show that the opposite is also true, namely, that the conformational state of the arms does not significantly influence the spectra of the stem. Subtracting the spectra of the dA_{15}:dT_{15} duplex measured between 10°C and 60°C from those of a solution containing $d(A_{15}G_{15})$ and dT_{15} yields the spectra of the stem shown in Fig. 2 *C I*. Increasing the temperature causes relatively minor changes in these difference spectra. The decrease of ~5 mdeg in the amplitude of the maxima at 257 nm as the temperature is increased is similar to the magnitude of the change observed in the absence of the complementary strand. Because only minor differences in the spectra of the stem are observed for frayed wires with either single-stranded or duplex arms, the spectroscopic characteristics of the stem are apparently independent of the conformational state of the arms.

We anticipated that the behavior of the two structural domains would be independent of the sequence of the arm of the frayed wire. The spectrum of an oligonucleotide containing thymine and adenine residues in the arms and the spectrum of an oligonucleotide with the same sequence as the arm were measured. Fig. 5 shows the resulting stem spectrum recovered for d(AAATAATAAATAAAAT AAG$_{15}$) and d(TTATTTTTATTTATTATTTG$_{15}$) frayed wires alone and bound to the oligonucleotide complementary to its arm. As expected, neither increasing the arm

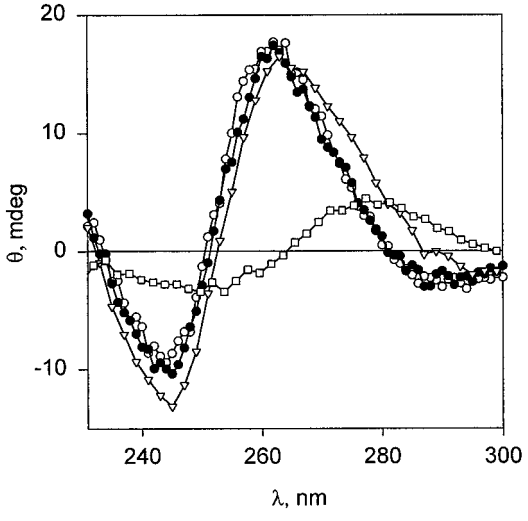

FIGURE 3 CD spectra of $d(A_{15}G_{15})$ frayed wires (○), $d(A_{15}G_{15})$:dT_{15} mixture (▽), dT_{15} (□), and a difference spectrum calculated by subtraction $d(A_{15}G_{15})$:dT_{15} − dT_{15} (●). Spectra were collected at 60°C.

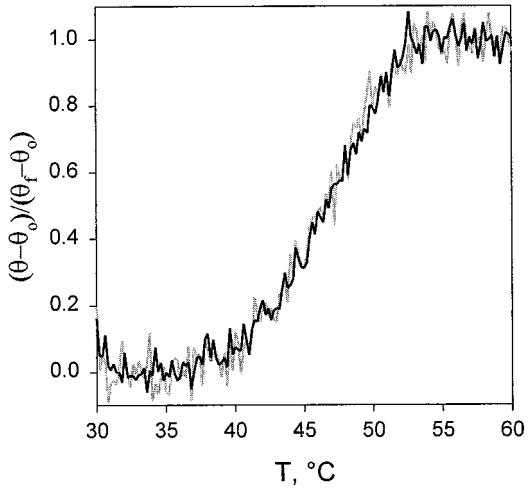

FIGURE 4 Helix-coil transition curves for $d(A_{15}G_{15})$:dT_{15} (*black*) and dA_{15}:dT_{15} (*gray*) complexes measured by recording the CD signal at 246 nm while increasing the temperature by 0.6°C/min.

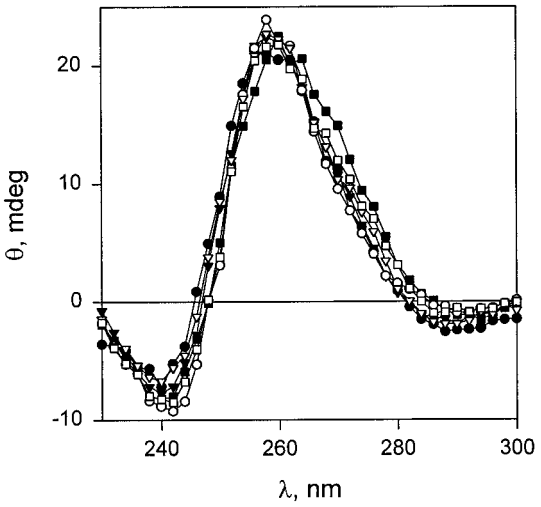

FIGURE 5 The stem spectra for the three types of frayed wires alone (*open symbols*) and upon binding of oligonucleotides complementary to the arms (*closed symbols*). *Circles*, $d(A_{15}G_{15})$; *triangles*, d(AAATAATA-AATAAAAATAAG$_{15}$); *squares*, d(TTATTTTTATTTATTATTTG$_{15}$).

length to 19 nucleotides nor the introduction of thymine residues alters the spectrum of the stem.

Solubility of frayed wires

In an effort to obtain spectra of poly(dG) in the presence of Mg^{2+} with which we could directly compare the spectra of the stem, we investigated the spectroscopic properties of dG_{30} and $d(AG_{15})$. These oligonucleotides aggregate in solutions containing Mg^{2+}, as is manifested by a decrease in absorbance at the 253-nm maximum. Interestingly, the complex formation is also accompanied by an increase in the signal at longer wavelengths. Because DNA is transparent to light in the 300–320-nm spectral region, the presence of the nonzero signal at the long wavelength part of the spectra must arise from light scattering by the solution, indicating the presence of large aggregates. Indeed, gel electrophoresis experiments showed that the high-molecular-weight aggregates of $d(AG_{15})$ predominate and precluded entry of the aggregates into the polyacrylamide gel (data not shown). We also observed that the intensity of the OD spectra of dG_{30} and $d(AG_{15})$ decreased with time and that the loss of intensity could be accelerated by centrifugation at 10,000 × *g*, implying that the aggregates are precipitating.

However, the spectroscopic signal for $d(A_{15}G_{15})$ did not decrease with time, nor was there a significant change after centrifugation under the same conditions. Apparently the arms of the frayed wire inhibit precipitation. This idea was investigated further by carrying out similar experiments on two other oligonucleotides, $d(A_5G_{15})$ and $d(A_{10}G_{15})$. The data in Table 1 show the results of incubation of the four oligonucleotides in Mg^{2+}-containing solution under ambient conditions and with centrifugation. In aqueous solutions containing 5 and 20 mM $MgCl_2$, there is a clear trend toward less precipitation with increasing arm length.

Spectroscopic properties of $d(A_{15}G_{15})$ in the presence of Na^+ and Et_4N^+

The CD spectra for the frayed wire stem in the presence of Mg^{2+}, Na^+, and Et_4N^+ are presented in Fig. 6 along with the spectrum of $d(AG_{15})$. Incubation of $d(A_{15}G_{15})$ in Na^+-containing solutions results in enhancement of the intensity of the positive band in the stem spectrum and a shift to longer wavelengths. The sodium-induced stem spectrum is identical to the spectrum of $d(AG_{15})$ obtained under the same conditions. However, in the presence of Mg^{2+}, the spectrum recorded for $d(AG_{15})$ differs from the stem spectrum, displaying an almost twofold decrease in the amplitude of the positive band and a shift toward longer wavelengths (data not shown).

The stem spectrum for the frayed wire grown in the presence of Et_4N^+ displays a decrease in the amplitude of the peak accompanied by a shift toward shorter wavelengths; the negative band becomes shallower. However,

TABLE 1 The effect of the arm length on the solubility of frayed wires

	ΔOD after incubation in 5 m*M* $MgCl_2$ at RT		ΔOD after centrifugation in 5 m*M* $MgCl_2$ at RT	ΔOD after incubation 20 m*M* $MgCl_2$ at RT	ΔOD after centrifugation in 20 m*M* $MgCl_2$ at RT	ΔOD after centrifugation following incubation for 3 days at 4°C
	253 nm	320 nm	253 nm	253 nm	253 nm	253 nm
$d(AG_{15})$	−17%	Increase	−73% 3 × 5 min × 10^4 g	—	—	—
$d(A_5G_{15})$	Unchanged	Unchanged	−5% 1 × 5 min × 10^4 g; 2 × 20 min × 1.2 × 10^4 g	−1.3%	−24% 1 × 2 min × 10^4 g	−16% 1 × 10 min × 10^4 g
$d(A_{10}G_{15})$	Unchanged	Unchanged	Unchanged 1 × 5 min × 10^4 g; 2 × 20 min × 1.2 × 10^4 g	−1.4%	−7% 1 × 2 min × 10^4 g	−10% 1 × 10 min × 10^4 g
$d(A_{15}G_{15})$	Unchanged	Unchanged	Unchanged 1 × 5 min × 10^4 g; 2 × 20 min × 1.2 × 10^4 g	−1%	−8% 1 × 2 min × 10^4 g	−3% 1 × 10 min × 10^4 g

%, (change in OD)/(initial OD).

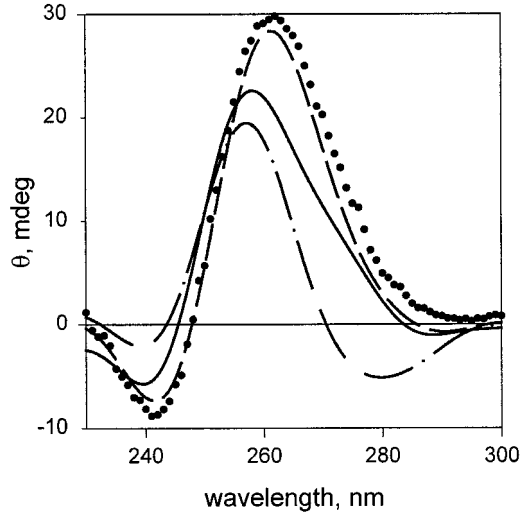

FIGURE 6 CD difference spectra representing the guanine stem of $d(A_{15}G_{15})$ frayed wires in the presence of 5 mM $MgCl_2$ (——), 100 mM NaCl (– – –), and 100 mM Et_4NCl (– · –). Also shown is the spectrum of $d(AG_{15})$ after incubation in 100 mM NaCl (●). Spectra were collected at 20°C.

the most distinguishing feature of the stem spectrum is the appearance of a new negative band at ~278 nm. This band was attributed to the presence of guanines in the single-stranded conformation (Gray and Bollum, 1974).

DISCUSSION

Single-stranded arms and guanine stem contribute independently to the spectra of frayed wires

Frayed wires are macromolecular aggregates formed by self-assembly of oligonucleotides with several consecutive 3′-terminal guanines such as $d(A_{15}G_{15})$ or $d(T_{15}G_{12})$. The spectroscopic experiments undertaken in this study were designed to provide insight into the conformation of DNA frayed wires. We have proposed that frayed wires consist of two distinct structural domains, namely single-stranded arms formed by the bases at the 5′ end and a stem formed by aggregated guanines at the 3′ end. Our original evidence for the proposed structure came from electrophoresis experiments in which the aggregated structures formed by $d(A_{15}G_{15})$ are retarded more in the presence of dT_{10} but not in the presence of dC_{10}.

The data presented show that upon subtraction of the CD spectrum of dA_{15} from the spectrum of $d(A_{15}G_{15})$, one obtains a spectrum of the stem that closely resembles the published spectra of poly(dG). "Decorating" the single-stranded arms with dT_{15} does not alter this property; the stem spectrum obtained upon subtracting the CD spectrum of the duplex, dA_{15}:dT_{15}, from the spectrum of $d(A_{15}G_{15})$:dT_{15} also resembles the stem spectrum of $d(A_{15}G_{15})$ and that of poly(dG) (Gray and Bollum, 1974; Marck and Thiele, 1978). These results offer additional evidence that the stem region is made up of complexes between guanine residues and that the dA_{15} arms behave spectroscopically like the free oligonucleotide dA_{15}. Thus the two structural domains inferred from the electrophoresis experiments are spectroscopically distinct and behave independently of each other.

The changes observed in the CD and OD spectra of $d(A_{15}G_{15})$ and $d(A_{15}G_{15})$:dT_{15} with increasing temperature reflect conformational transitions in the arms. The spectra at different temperatures of $d(A_{15}G_{15})$ alone in solution are consistent with the loss of secondary structure in the single-stranded A_{15} region. The spectra of the stem, i.e., the spectrum obtained upon subtraction of the spectrum of dA_{15} from that of $d(A_{15}G_{15})$, remain essentially unchanged from 10°C to 60°C. This result is consistent with our previous finding that frayed wires are stable, even in denaturing polyacrylamide gels (7 M urea, 55°C).

We conclude that the conformation of the arm does not exert a strong influence on the structure of the stem, nor does the stem alter the properties of the complexes formed with the arm. The helix-coil transition temperature is the same for $d(A_{15}G_{15})$:dT_{15} complexes and dA_{15}:dT_{15} duplex. This result is surprising, because one might expect the adenines near the junction with guanines to be conformationally constrained by the presence of the stem and that this would influence the stability of the complexes with dT_{15}. In addition, irrespective of the actual guanine-guanine interactions within the stem, the charge density at the 3′ end of the arms of a frayed wire is expected to be significantly greater than the charge density at the 3′ end of dA_{15}. However, despite these considerations, the spectral and the thermal data indicate that there is very little interaction between the stem and arm regions of a frayed wire.

The conformation of the stem

The stem spectra we report here are similar to the spectrum of the Na^+-induced self-complexed form of poly(dG). There is a positive maximum centered at ~257 nm and a trough at ~240 nm; the ratio of the intensities of these 257 nm and 240 nm peaks is ~3 (Gray and Bollum, 1974; Marck and Thiele, 1978). The oligonucleotide $d(TG_4T)$, as well as a number of telomeric oligonucleotides, exhibits similar spectra (Lu et al., 1993; Balagurumoorthy et al., 1992; Hardin et al., 1991; Marotta et al., 1996). Based on the demonstration by x-ray crystallography that the self-complementary interactions of $d(TG_4T)$ arise from guanine tetraplexes, this CD signal has been attributed to the formation of G-tetrads (Laughlan et al., 1994). The CD spectra of guanine-rich DNA reported in the literature vary with the sequence, length, and position of guanine tracks, and thus reflects the variety of conformational arrangements that these molecules can adopt. This includes parallel tetraplex, antiparallel dimers, interquadruplex aggregates (Chen, 1995, 1997), as well as complex structures resulting from the coexistence of species with different strand geometries

(Balagurumoorthy et al., 1992; Hardin et al., 1991; Marotta et al., 1996).

Because the conformation adopted by the complexes is dependent on the type of cation in solution, presumably the type of cation may also affect the CD spectra. In the present study we have focused on the spectroscopic properties of the multistranded DNA complexes formed in the presence of magnesium. We had previously demonstrated the role of divalent cations in the formation of frayed wires of $d(A_{15}G_{15})$ by electrophoretic techniques (Protozanova and Macgregor, 1996). Magnesium and calcium ions were shown to stabilize higher molecular weight aggregates better than alkali ions.

The stem spectrum of frayed wires formed in the presence of 100 mM Na^+ resembles the spectrum of a tetraplex with a parallel arrangement of strands (Lu et al., 1992, 1993) and the spectrum of $d(AG_{15})$ presented in this work. Antiparallel four-stranded complexes are characterized by the appearance of a positive band at ~295 nm (Lu et al., 1993; Balagurumoorthy et al., 1992); there is no evidence in our data for the formation of these structures. Gel electrophoresis experiments have shown that high sodium ion concentrations enhance the formation of the second band in the frayed wire ladder to the extent that these species account for up to 40% of the total intensity in the lane (Protozanova and Macgregor, 1996). In our previous publication, we attributed this band to a structure formed by two molecules of oligonucleotide. Because of the absence of the spectroscopic evidence for antiparallel tetraplexes, we propose that this band arises from formation of a G:G duplex. This proposal is further supported by the ability of dC_{10} and other cytosine-rich oligonucleotides to interact with the dimeric species, most likely through the formation of a triple-stranded complex.

The CD spectrum of single-stranded guanine can be inferred from the spectra of acetylated poly(dG), which has not been observed to form aggregates; the spectra of dG_5 at elevated temperatures (Gray and Bollum, 1974); and the spectra of dG_4 in the presence of tetraethylammonium ions (Thomas et al., 1984). The distinguishing spectral features of the single-stranded form are the presence of a negative band centered at ~277 nm, a decrease in the intensity of the trough at ~240 nm, and a shift of the peak to the shorter wavelengths. Fig. 6 shows the spectrum of the stem of $d(A_{15}G_{15})$ in the presence of Et_4N^+; it displays the features of single-stranded guanine-rich DNA. Also shown in Fig. 6 are the spectrum of the stem of $d(A_{15}G_{15})$ in 100 mM NaCl and the spectrum of $d(AG_{15})$ in 100 mM NaCl. It is apparent that the stem spectrum in Mg^{2+} is not the same as any of these spectra. This suggests two possibilities for the origin of the spectrum of the stem of $d(A_{15}G_{15})$ in the presence of magnesium ions. The stem of the aggregated species may be in two conformations that are nearly energetically equivalent, for example, tetraplex and single-stranded, and the CD spectrum is the sum of the contributions of the two states. Alternatively, the stem may be in a structure that does not correspond to either the tetraplex or the single-stranded conformation. It does not appear that our present data allow us to differentiate between these two possibilities.

The role of Mg^{2+} in the supramolecular aggregation of guanine-rich oligonucleotides

Relative to the spectrum of $d(A_{15}G_{15})$, in the presence of Mg^{2+} the main positive band in the CD stem spectrum of $d(AG_{15})$ is approximately half as intense and is shifted to longer wavelengths (data not shown). Similar spectroscopic changes induced by Na^+ and Mg^{2+} were reported by Hardin et al. (1991) in their study of the influence of the type of the cation on complex formation by $d(AG_7AGAG_6AG_6)$. Note, however, that in their investigation they found that another oligonucleotide, with a higher nonguanine nucleotide content, displayed a smaller difference between the CD spectra in solutions containing sodium and magnesium ions.

Absorbance spectra for the stem and $d(AG_{15})$ are also different, with $d(AG_{15})$ having a long wavelength shoulder and a decrease in the amplitude of the peak at 253 nm (Table 1). Similar spectroscopic changes reported by Chen (1995) have been attributed to the supramolecular self-association of $d(CGG)_4$. The aggregation of these oligonucleotides arises from C^+:C interactions between cytosines looped out of the guanine quadruplex stem. Association of quadruplexes in a lateral fashion accounts for the formation of very large aggregates and, consequently, an apparent increase in the absorption due to light scattering.

In the context of the current study, the spectral differences between induced complexes formed by $d(A_{15}G_{15})$ and $d(AG_{15})$ in the presence of Mg^{2+} may be a consequence of the adenine residues at 5′ end of $d(A_{15}G_{15})$, which interfere with a magnesium-facilitated interaction between individual complexes and subsequent formation of very large complexes. The role of single-stranded arms in impeding precipitation is further supported by the behavior of these oligonucleotides upon centrifugation. The data in Table 1 show a clear correlation between the length of the arm of a frayed wire and its solubility. After centrifugation for 45 min at $10^4 \times g$, the extent of precipitation of $d(A_{15}G_{15})$, as monitored by the decrease in OD at 253 nm, was negligible; however, almost no $d(AG_{15})$ remained in solution after 15 min of centrifugation at $10^4 \times g$. We also observed a correlation between the magnesium concentration and the rate of precipitation. Increasing the magnesium concentration to 20 mM led to increased precipitation of the frayed wires with intermediate arm lengths, $d(A_5G_{15})$ and $d(A_{10}G_{15})$.

The role of Mg^{2+} in the formation of multistranded structures by oligonucleotides with long or multiple runs of guanines has been emphasized by us and others (Protozanova and Macgregor, 1996; Marotta et al., 1996; Chen, 1997; Marsh et al., 1995). When visualized by scanning probe microscopy, G-wires, the polymeric complexes of $d(G_4T_2G_4)$, appear to form longer aggregates after incubations in Mg^{2+}-containing solutions (Marsh et al., 1995).

Marotta et al. (1996) report that the formation of high-molecular-weight species arising from a variety of oligonucleotides with a $G_4T_2G_4$ motif at their 3′ end is greatly enhanced when Mg^{2+} is present together with alkali cations. For oligonucleotides with short nonguanine 5′-end portions, the ellipticity of the positive band decreased with time upon incubation in solutions containing both potassium and magnesium ions. This result agrees with our finding that the presence of magnesium facilitates precipitation of frayed wires with short arms. Chen has proposed that magnesium ions stabilize multistranded architectures because of their ability to neutralize the electrostatic repulsion of the phosphate groups, as well as though promotion of lateral expansion via bridging between individual quadruplexes (Chen, 1997). Such lateral association accounts for the appearance of the so-called ψ-type CD spectrum for $d(TGG)_4$, characteristic of the formation of highly ordered, condensed DNA. In our case, magnesium ions facilitate the self-association that leads to the formation of frayed wires and, for the oligonucleotides/frayed wires with short arms, it promotes the further aggregation that results in precipitation. Longer arms presumably pose a structural obstacle for aggregation between frayed wires. Thus the arms, although independent of the stem of the frayed wire, are essential for maintaining the solubility of these large multimolecular complexes.

We thank Dr. Avi Chakrabartty for the use of the CD instrument in his laboratory.

This work was supported in part by a grant from Glaxo Wellcome Canada. EP is a PMAC-MRC Scholar.

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