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Dimerization of the guanine-adenine repeat strands of DNA

Michaela Vorlícková*, Iva Kejnovská, Jirí Kovanda and Jaroslav Kypr

Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, CZ-61265 Brno, Czech Republic

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ABSTRACT

Jovin and co-workers have demonstrated that DNA strands containing guanine-adenine repeats generate a parallel-stranded homoduplex. Here we propose that the homoduplex is a dimer of the ordered single strand discovered by Fresco and co-workers at acid pH. The Fresco single strand is shown here to be stabilized in aqueous ethanol where adenine is not protonated. Furthermore, we demonstrate that the strands dimerize at higher salt concentrations without significantly changing their conformation, so that the dimerization is non-cooperative. Hence, the Jovin homoduplex can form through a non-cooperative dimerization of two cooperatively melting single strands. The available data indicate that the guanines stabilize the Fresco single strand whereas the adenines cause dimerization owing to their known intercalation or clustering tendency. The guanine-adenine repeat dimer seems to be a DNA analog of the leucine zipper causing dimerization of proteins.

INTRODUCTION

DNA strands containing guanine-adenine repeats self-associate (1,2). The self-associations include a parallel-stranded homoduplex (3,4) and various antiparallel homoduplexes (5–7). These unusual homoduplexes, which require no pyrimidine base to be stable, are also adopted by related sequences (8–11). The $(G,A)_n$ strands participate in triplexes as well (12–15). Recently, $(AG)_{10}$ has been reported to form foldbacks at low ionic strength and neutral pH, with the ability to self-associate at higher ionic strengths (16) through guanine tetrads (17). In this structure, the adenines were intercalated but did not hydrogen bond to other bases.

The $(GA)_n$ sequences also fold in a single-stranded, cooperatively melting conformer at acidic pH (18–20). Fresco and co-workers (20) have produced a molecular model of this acid-stabilized single strand in which the bases are not stacked and do not hydrogen bond to each other, but instead the structure is stabilized by hydrogen and ionic bonds between the phosphodiester backbone and the protonated adenine lying approximately parallel to the helix axis. Longer $(A,G)_{20,30}$ tracts form a parallel-stranded homoduplex containing $A^+ \cdot A^+$ and G·G pairs (21).

Structural peculiarities of $(G,A)_n$ strands are of biological interest because these sequences are abundant in genomes (22), including at centromeres, where they seem to confer specific conformational properties on DNA (23,24). Related $(GAA)_n$ repeats are associated with the trinucleotide repeat expansion disease Friedreich's ataxia (25,26). Conformational properties of DNA strands containing guanine and adenine have recently been reviewed (27).

In this paper we show that the Fresco ordered single-stranded structure may be found not only in acidic but also in ethanolic solution where adenine is not protonated. Furthermore, the Fresco single strands are shown to dimerize at higher ionic strengths both in acid and ethanol solution, without substantially changing the conformation. The resulting homoduplexes are essentially the same as the homoduplex previously described by Jovin and co-workers (3).

MATERIALS AND METHODS

$(GA)_{10}$ was synthesized, purified and characterized as described previously (28) and the lyophilized oligonucleotide was dissolved in 1 mM Na phosphate, 0.3 mM EDTA, pH 7. Concentrations were determined at 25°C using a UNICAM 5625 UV/Vis spectrometer and the molar extinction coefficient 11 820/M cm at 255 nm. Buffer conditions were altered by adding either Na phosphate or Britton-Robinson buffer. Salt concentrations were increased by adding concentrated salt solutions or known weights of solid salts directly to the cells (with appropriate corrections for sample volume increases). pH was varied by adding HCl, NaOH or a buffer component to the cells, where it was directly measured using a Radelkis pH meter and a Metrohm electrode.

CD spectra were collected using the Jobin-Yvon Mark IV and Mark VI spectrometers in 0.1, 1 and 5 cm path length Hellma cells placed in a thermostatted holder. Ellipticity is expressed as per M cm, the molarity being related to the nucleoside residues in the DNA samples. DNA concentrations were such as to give absorbances of 0.5–0.8 at 260 nm, in order to minimize noise in the CD measurements.

Non-denaturing polyacrylamide gel electrophoresis utilized a thermostatted submarine apparatus (SE-600; Hoefer Scientific, San Francisco, CA). Gels (20%, 29:1 monomer/bis ratio), 14 × 16 × 0.1 cm in size, were run for 20 h at 70 V (~5 V/cm) and 0°C.

The gels were stained with Stains-All. Densitometry was performed using the Personal Densitometer SI, Model 375-A (Molecular Dynamics, Sunnyvale, CA). The DNA markers $(GT)_{10}$ and $(AC)_{10}$ were synthesized by IDT and bought from East Port (Prague, Czech Republic).

RESULTS

The CD spectrum of the GA homoduplex has a dominant positive band at 265 nm (3,11; Fig. 1). This homoduplex is stable between

*To whom correspondence should be addressed. Tel: +420 541517198; Fax: +420 541240497; Email: mifi@ibp.cz

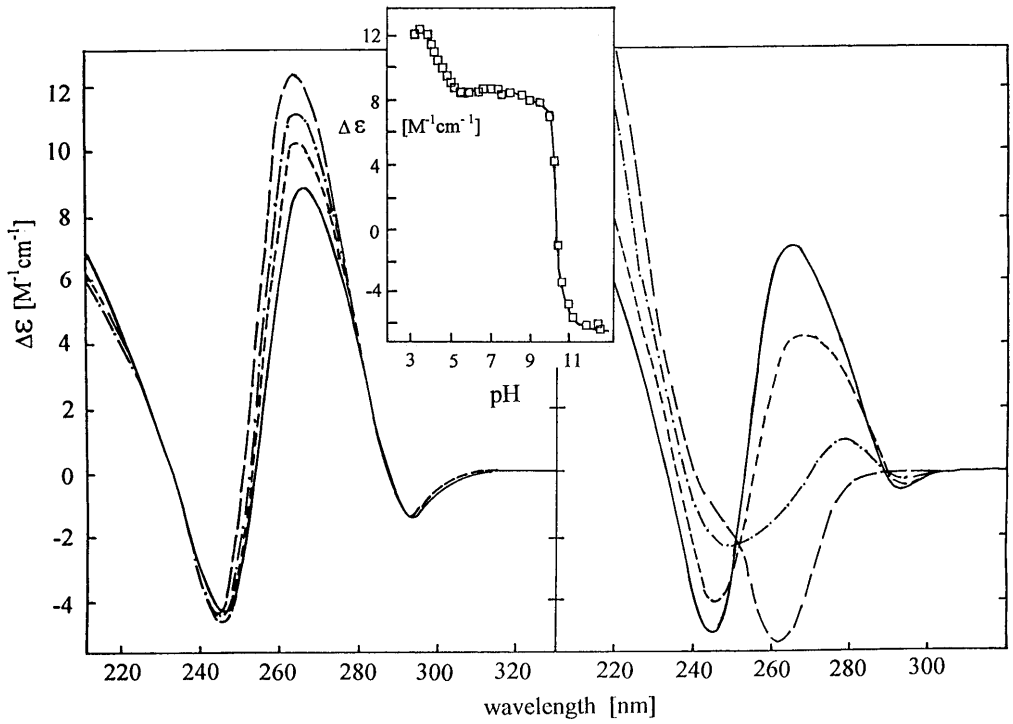

Figure 1. CD spectra of $(GA)_{10}$ dissolved in various Britton-Robinson buffers + 0.1 M KCl and measured at 2.7°C in 1 cm path length cells.The values of pH were: (left) 4.0 (––), 4.3 (– . –), 4.7 (- - -) and 5.2 (—); (right) 9.9 (—), 10.3 (- - -), 10.5 (––) and 11.0 (– –). (Insert) The pH dependence of the ellipticity of $(GA)_{10}$ at 265 nm.

pH 5 and 10 (Fig. 1). Above pH 10 the homoduplex denatures in a two-state cooperative process and below pH 5 an ordered acid conformer is seen whose CD spectrum exhibits a still more pronounced positive band (Fig. 1). The acid conformer is more thermostable than the neutral homoduplex and electrophoretic mobility indicates that the acid conformer is also a homoduplex (Fig. 2), which was also the finding of a separate study on $(GA)_{20}$ at pH 4.6 (5). These results appear to be at odds with literature claims of the ordered single stranded conformer in acid pH (18–20), which motivated us to study alternating $(GA)_n$ sequences in more detail.

Salt induction of the neutral homoduplex of $(GA)_{10}$

Figure 3 shows the CD spectra and the UV absorption spectra of $(GA)_{10}$ undergoing a NaCl-induced transition from a denatured single strand to the neutral homoduplex. As usual for DNA, the process is hypochromic (Fig. 3, right and insert) and is promoted by increasing oligonucleotide concentration (Fig. 3, insert). Various cations stabilize the neutral pH homoduplex in a charge-dependent manner, since their effectiveness decreased in the order spermine > hexaminecobalt > magnesium > KCl ≈ NaCl ≈ LiCl > $NaClO_4$ > CsF (not shown).

Acid pH induction of the Fresco single strand

At low salt and neutral pH, $(GA)_{10}$ is denatured and undergoes a fast (kinetics shorter than minutes), reversible, two-state and cooperative acid-induced transition into an ordered conformer. The final CD spectrum is similar to that of the neutral homoduplex (compare Fig. 4 with Fig. 3, left). However, the positive band at 262 nm is stronger and the negative band at 240 nm

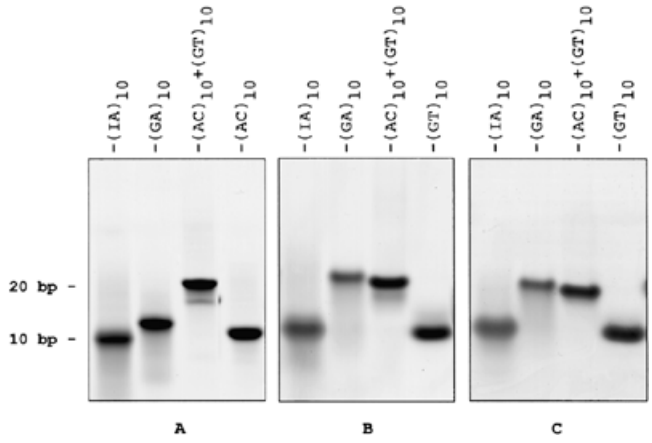

Figure 2. PAGE of $(GA)_{10}$ run at 0°C in: (**A**) 1 mM Na phosphate, 0.3 mM EDTA, pH 7; (**B**) Britton-Robinson buffer containing 0.1 M NaCl, pH 7; (**C**) Britton-Robinson buffer containing 0.1 M NaCl, pH 4. The markers include the single-stranded DNA 20mers $(IA)_{10}$, $(AC)_{10}$, $(GT)_{10}$ and the duplex $(AC)_{10}{\cdot}(GT)_{10}$.

is weaker, the amplitude (at 262 and 240 nm) ratio being 3.5 for the acid form and 1.7–2 for the neutral homoduplex. This indicates a base tilt in the acid conformer (29). The acid-induced transition results in UV hyperchromicity caused by reduced base stacking. Changes in both CD and UV spectra are unaffected by a 50-fold dilution of the oligonucleotide (Fig. 4, insert), implying a monomolecular structure. These results are consistent with the Fresco model for the single-stranded acid-stabilized conformer (20).

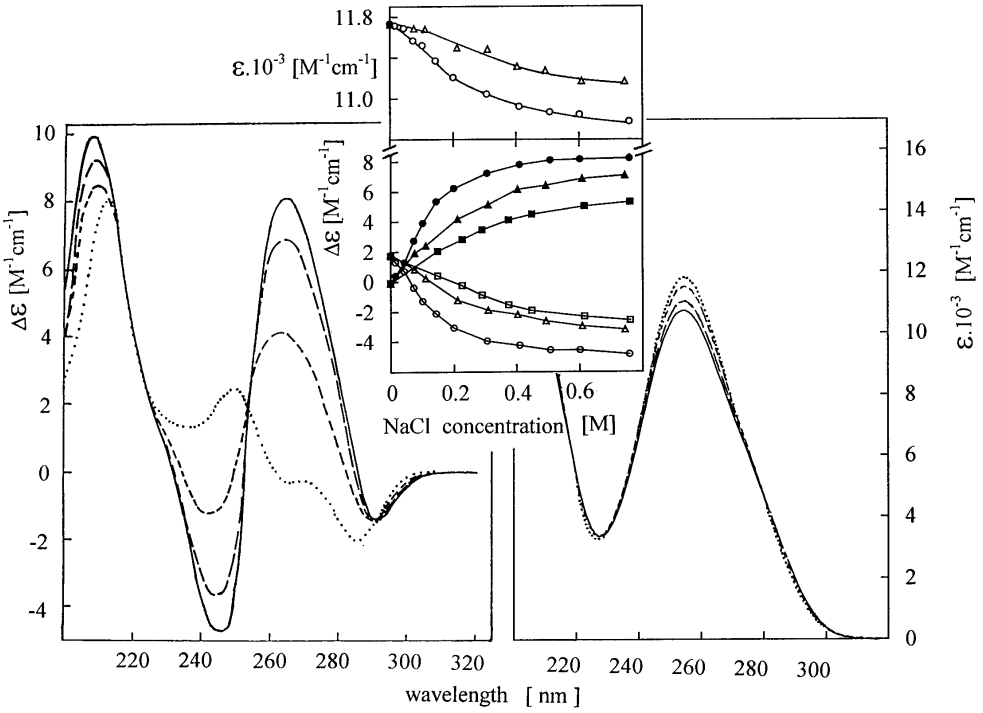

Figure 3. NaCl-induced changes in the (left) CD spectra and (right) UV absorption spectra of $(GA)_{10}$, both measured in 0.1 cm path length cells at 22°C. The buffer contained 10 mM Na phosphate, 0.3 mM EDTA, pH 7, and NaCl, whose concentrations were 0 (····), 0.1 (- - -), 0.3 (– –) and 0.75 M (—). (Insert) $(GA)_{10}$ homoduplex induction by NaCl at 0.59 (circles), 0.045 (triangles) and 0.008 mM (squares) concentrations of the $(GA)_{10}$ nucleoside residues measured in 0.1, 1 and 5 cm path length cells. (Top) Absorbance at 255 nm; (bottom) ellipticity at 265 (closed symbols) and 245 nm (open symbols).

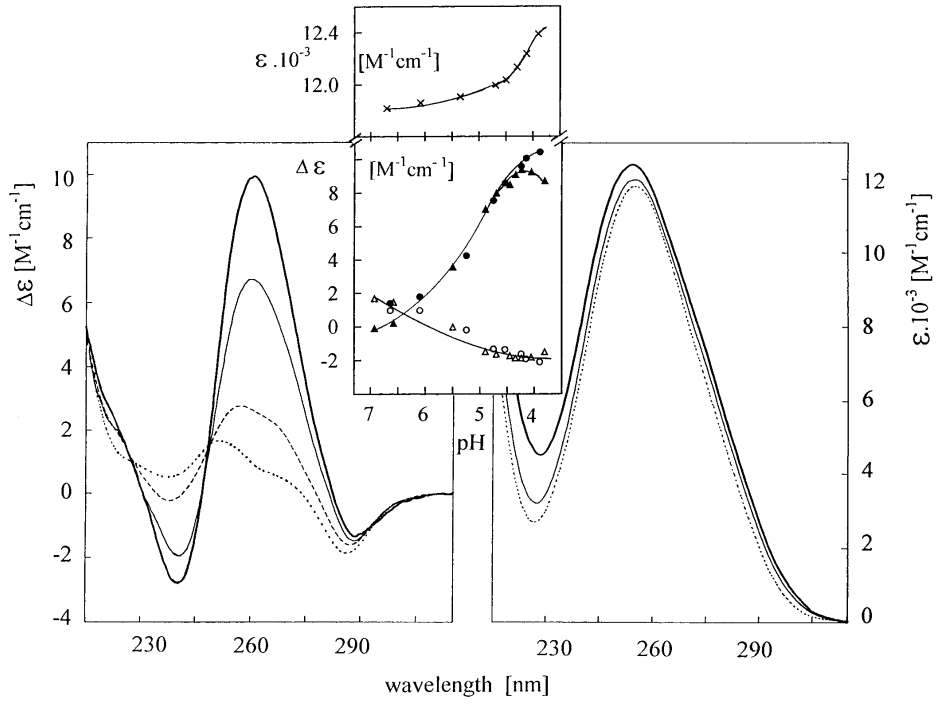

Figure 4. pH-induced changes in the (left) CD spectrum and (right) the UV absorption spectrum of $(GA)_{10}$. All measurements were carried out in 1 cm path length cells at 22°C. The oligonucleotide was originally dissolved in 1 mM Na phosphate, 0.3 mM EDTA, pH 7, to which HCl was added to get the following values of pH: 6.7 (····), 5.4 (- - -), 4.7 (—) and 3.9 (bold). (Top insert) pH dependence of the absorbance of $(GA)_{10}$ at 255 nm. (Bottom insert) pH dependences of the ellipticity of $(GA)_{10}$ at 262 (closed symbols) and 242 nm (open symbols), measured at 0.62 (triangles) and 0.008 mM (circles) nucleoside residue concentrations of $(GA)_{10}$.

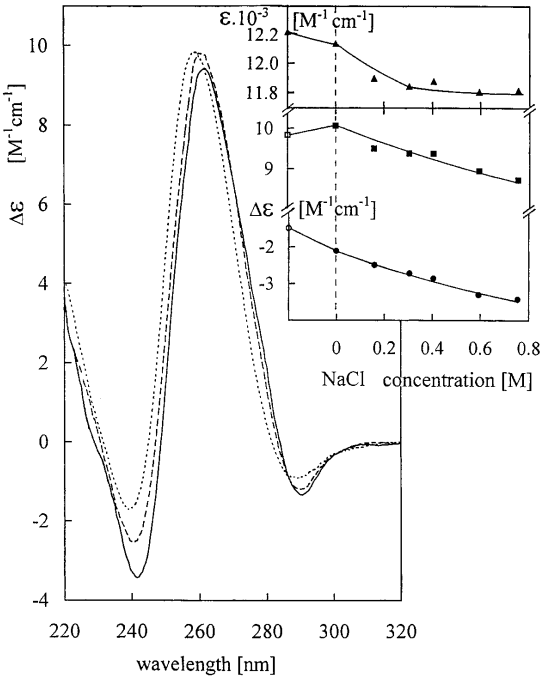

Figure 5. Spectral changes during the NaCl-induced transition from the acid single strand of $(GA)_{10}$ to its acid homoduplex. 1 mM Na phosphate + 0.3 mM EDTA, pH 3.8 (····); Britton-Robinson buffer, 0.16 M NaCl, pH 3.8 (- - -); Britton-Robinson buffer, 0.75 M NaCl, pH 3.8 (—). (Insert) Salt dependences of (top) the absorption at 255 nm and (bottom) the ellipticity at 259 (squares) and 240 nm (circles) of $(GA)_{10}$ in Britton-Robinson buffer, pH 3.8. Open symbols correspond to values measured for $(GA)_{10}$ in 1 mM Na phosphate, 0.3 mM EDTA, pH 3.8. The measuremens were carried out in 1 cm path length cells at 22 °C.

The neutral homoduplex to acid homoduplex transition

Electrophoretic migration (Fig. 2) shows that $(GA)_{10}$ is a homoduplex at low temperature in neutral as well as acid pH buffer containing 100 mM NaCl or KCl. The corresponding CD spectra of the neutral and acid homoduplexes (Fig. 1) differ only by an enhanced positive band, but the CD spectrum shape remains unchanged. The transition between the two homoduplexes is reversible and fast, which hardly appears compatible with significant differences in their structure. Consequently, the duplexes seem to differ only by adenine protonation inducing but minor conformational modifications in the acid homoduplex.

The transition from the acid single strand to the acid homoduplex

It was surprising that the amplitude of the diagnostic CD band at 265 nm was almost independent of ionic strength at acidic pH because $(GA)_{10}$ was single stranded at the lower, but homoduplex at the higher ionic strength. The detailed dependence reflecting the transition from the acid single strand to the acid homoduplex, however, exhibited only small, gradual changes in the CD spectrum and mild hypochromicity (Fig. 5). Hence the homoduplex seems to be a simple side-by-side association of two Fresco single strands.

The Fresco single strand of $(GA)_{10}$ is stabilized by ethanol

Ethanol is known to switch DNA into non-B conformers and we thought it would be interesting to determine its effects on $(GA)_{10}$. Remarkably, addition of ethanol to denatured $(GA)_{10}$ induced changes in the CD spectra like acidic pH (Fig. 6). The transition was cooperative and two-state over 5–25% ethanol and was complete at ~40% ethanol. Upon dilution, the changes rapidly reversed and a 50-fold change in the oligonucleotide concentration had no significant effect (Fig. 6, insert), implying that a monomolecular transition is being observed. Hyperchromic changes in the UV spectrum were further consistent with the hypothesis that ethanol acted like acid to stabilize the Fresco fold. Interestingly, increasing the pH to 9.5 did not hinder the transition, implying that adenine protonation plays no necessary role in the formation of this structure. Methanol induced the same effects as ethanol, while trifluorethanol was less effective (not shown).

Addition of ethanol at higher salt concentrations, which partially stabilized the homoduplex of $(GA)_{10}$ in neutral aqueous solution, caused the same CD changes as seen for the single strands, but the required amounts of ethanol were smaller and the transitions were less cooperative (Fig. 7). If the initial salt concentration was such that the homoduplex was largely stable in the neutral aqueous solution, then addition of ethanol only caused a small, gradual increase in its CD bands. Irrespective of the initial ionic strength, all $(GA)_{10}$ samples converged to the same ellipticity values in ethanolic solutions (Fig. 7). This is the same result as obtained with acid to reinforce the point that the Fresco single strand and the Jovin homoduplex have essentially the same CD spectra (Fig. 8). Hence the homoduplex appears to be a simple dimer of two molecules of the ordered single-stranded conformer. The salt-induced dimerization of the ethanol-stabilized Fresco single strand caused a UV spectrum depression (Fig. 8) as in the case of salt-induced dimerization in acidic aqueous solution.

Thermostabilities of the $(GA)_{10}$ conformers

Figure 9 compares the thermal melting curves of the ordered single strand and homoduplex conformers of $(GA)_{10}$ stabilized by ethanol, salt and acid pH. The ethanol-induced single strand is the least stable. Interestingly, the thermostability of the $(GA)_{10}$ homoduplex is only slightly influenced by the presence of ethanol, indicating that the role of hydration is different in the stabilization of the Watson–Crick and Fresco–Jovin duplexes. Adenine protonation markedly influences the thermostability, but salt has little effect at acid pH. The low salt acid single strand melts at an almost identical temperature to the acid homoduplex in 160 mM NaCl (Fig. 9). Hence dimerization of the Fresco single strands contributes nothing to their thermostability.

DISCUSSION

The present paper demonstrates that the CD spectrum of the Fresco ordered single strand of $(GA)_{10}$ is essentially the same as that of the Jovin $(GA)_{10}$ homoduplex. This result implies that the homoduplex comes about by a side-by-side interaction of two ordered single strands and that dimerization does not require structural rearrangement of the single-stranded conformer. This gives rise to the curious situation that melting of the single-stranded conformer is cooperative while duplex formation is non-cooperative. This is exactly the opposite of Watson–Crick single strands and duplexes.

The idea that the homoduplex of $(GA)_{10}$ is a dimer of two ordered single strands is new as far as DNA is concerned, but there is an analogous protein structural motif, i.e. the two-stranded parallel coiled-coil of the leucine zipper (30,31), where the leucine side chains extrude from the participating α-helices

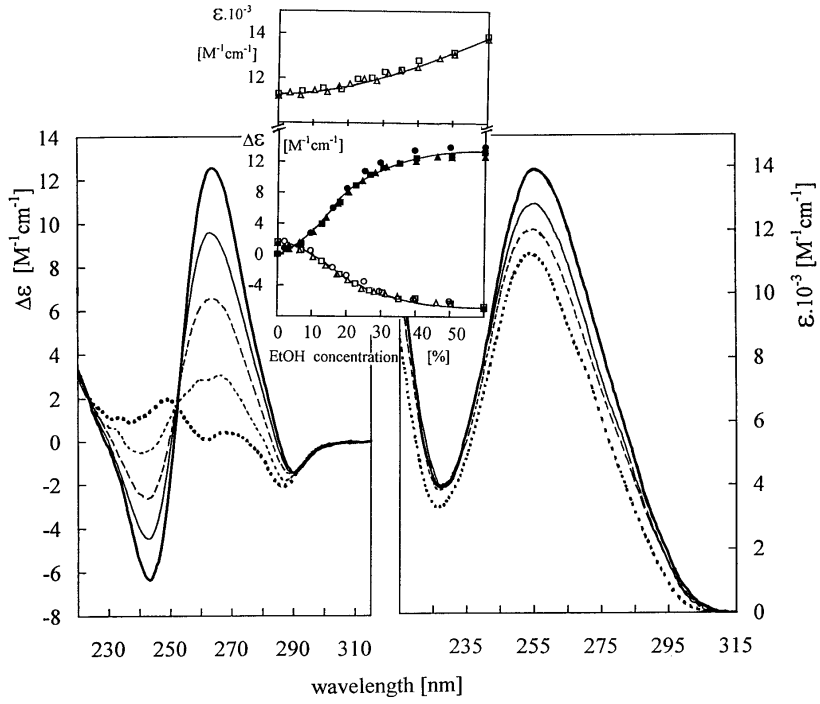

Figure 6. Ethanol-induced changes in the (left) CD and (right) UV absorption spectra of $(GA)_{10}$ originally dissolved in 1 mM Na phosphate, 0.3 mM EDTA, pH 7, to which ethanol was added to give: (left) 0 (····), 10 (- - -), 17 (– –), 24 (—) and 60% (bold); (right) 0 (····), 26 (- - -), 40 (—) and 60% (v/v) (bold) concentrations.The measurements were carried out at 0°C in 0.1 cm path length cells. (Inserts) Dependences on the ethanol concentration of (top) the absorbance at 255 nm and (bottom) the ellipticity at 261 (closed symbols) and 242 nm (open symbols). The measurements were carried out at 0.58 (triangles), 0.06 (squares) and 0.008 mM (circles) nucleoside residue concentrations of $(GA)_{10}$.

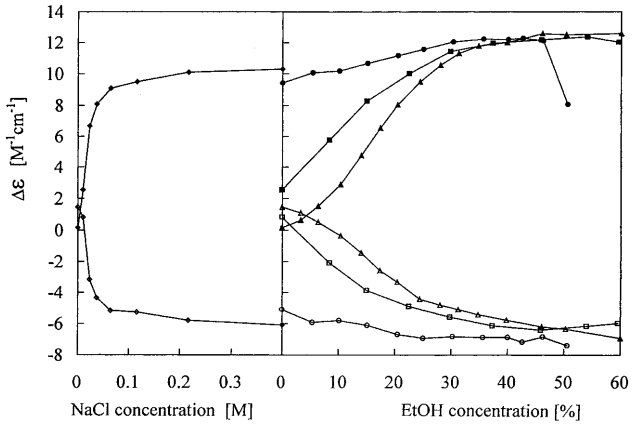

Figure 7. Ethanol-induced changes in the CD spectra of $(GA)_{10}$ homoduplexed to various extents by NaCl in the absence of ethanol. The measurements were carried out in 0.1 cm path length cells at 0°C. (Left) Ellipticity dependences of $(GA)_{10}$ on the concentration of NaCl at 0°C in the absence of ethanol. The oligonucleotide was originally dissolved in 10 mM Na phosphate, 0.3 mM EDTA, pH 7. (Right) Ellipticity dependences of $(GA)_{10}$ on the concentrations of ethanol in 1 mM Na phosphate, 0.3 mM EDTA, pH 7 (triangles), 10 mM Na phosphate, 0.3 mM EDTA, pH 7 (squares) and the same buffer plus 0.15 M NaCl, pH 7 (circles). The oligonucleotide precipitated from the latter solution at high concentrations of ethanol. In all of these experiments, the ethanol was added to the oligonucleotide samples as an aqueous solution containing the same buffer and salt concentrations as the oligonucleotide sample. The open and closed symbols correspond to the ellipticity at 245 and 265 nm, respectively.

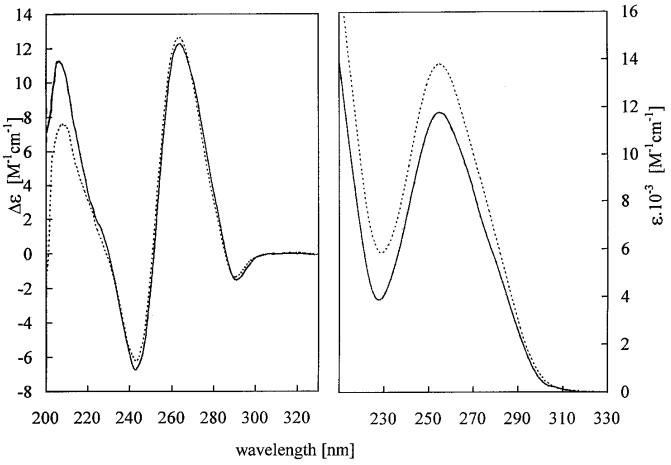

Figure 8. Comparison of the (left) CD and (right) UV absorption spectra of the ordered single strand of $(GA)_{10}$ in 0.54 mM Na phosphate, 0.16 mM EDTA, pH 7, 46% ethanol (····) and of the homoduplex of $(GA)_{10}$ stabilized by 10 mM Na phosphate, 0.30 mM EDTA, 0.16 M NaCl, pH 7, and 46% ethanol (—).

and significantly contribute to the dimerization. In the guanine-adenine repeat dimer, adenines perhaps play a similar role thanks to their remarkable intercalation or clustering tendency (32–34). This could account for the non-cooperative nature of the dimerization. Our preliminary experiments indicate that guanine is essential for stabilization of the Fresco single strand whereas Raman spectroscopy demonstrates that the adenines are loosely held in this structure and thus available for intercalation, to which adenine

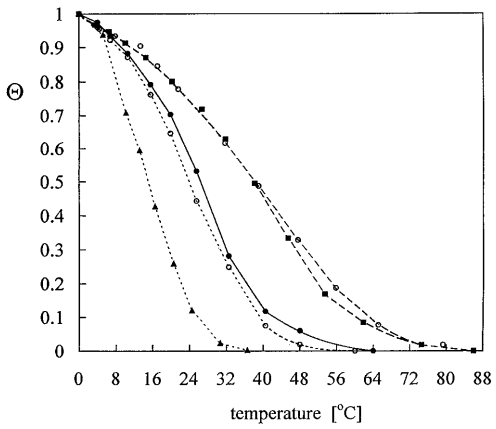

Figure 9. Comparison of the temperature dependences of the fraction Θ of non-denatured $(GA)_{10}$ measured in 42% ethanol, 0.6 mM Na phosphate, 0.17 mM EDTA, pH 7 (··▲··), 42% ethanol, 0.16 M NaCl, 10 mM Na phosphate, 0.3 mM EDTA, pH 7 (··○··), 0.16 M NaCl,10 mM Na phosphate, 0.3 mM EDTA, pH 7 (–●–), 1 mM Na-phosphate, 0.3 mM EDTA, pH 3.8 (--■--) and Britton-Robinson buffer, 0.16 M NaCl, pH 3.8 (--○--). Θ was calculated from the ellipticity values at 262 nm.

is particularly prone (32,33). Adenines mediate tertiary interactions in RNA (35) and they are likely to do the same in DNA (34).

The model also explains the effects of ethanol and acid. Both agents increase contact between the hydrophobic bases and the solvent. Thus it is not the protonation *per se*, but exposure of adenine to the solvent, which is required for stabilization of the Fresco single-stranded structure. Ethanol promotes UV crosslinking of DNA duplexes (36), which is consistent with a tendency of ethanol to expose photoreactive hydrophobic bases that may then mediate contacts between DNA duplexes (36). There are also other examples of interactions proposed to mediate DNA–DNA contacts (37–40).

The idea of exposed or flipped out bases, depending on the sequence context and its specific conformational properties, has wide biological implications because it is the central point of DNA repair, methylation of cytosine and adenine in DNA (41) and DNA recombination. Poly(GA)·poly(TC) duplexes have been shown to dimerize (42) and DNA duplexes containing $(GAA)_n$·$(TTC)_n$ repeats also interact (43–45) in line with our unpublished observations, demonstrating that dimerization is not restricted to $(GA)_n$ sequences. It seems that DNA interacts not only with proteins, but also with itself. Maybe the DNA–DNA interactions will explain why there are so many simple sequence repeats in eukaryotic genomes.

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