Divalent Zinc Cations Induce the Formation of Two Distinct Homoduplexes of a $d(GA)_{20}$ DNA Sequence†

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ABSTRACT: Homopurine DNA sequences are highly structurally polymorphic. In particular, $d(GA)_n$ DNA sequences are known to be capable of forming intramolecular foldbacks, bimolecular homoduplexes, and tetrastranded complexes. Counterions play a determinant role on the equilibria between the different structural conformers of $d(GA)_n$ sequences. In this paper, the effect of divalent zinc cations on the structure of a $d(GA)_{20}$ oligonucleotide has been analyzed by CD spectroscopy and polyacrylamide gel electrophoresis. Depending on the precise experimental conditions at which zinc is added, two distinct conformations of the $d(GA)_{20}$ oligonucleotide are stabilized. At neutral pH in the absence of zinc, $d(GA)_{20}$ is partially organized into intramolecular foldbacks and bimolecular homoduplexes [Casasnovas et al. (1993) *J. Mol. Biol. 233*, 671−681]. Under these conditions, addition of zinc results in the stabilization of the bimolecular homoduplex which is nonspecific for zinc since it is also stabilized by divalent magnesium cations, increasing ionic strength, or decreasing pH. Its CD spectrum is identical to that reported earlier for parallel-stranded $d(GA)_n$ homoduplexes [Rippe et al. (1992) *EMBO J. 11*, 3777−3786]. On the other hand, if zinc is added under conditions where the $d(GA)_{20}$ oligonucleotide is exclusively single-stranded, a different bimolecular homoduplex appears which is only observed in the presence of zinc. The zinc-specific duplex melts cooperatively, and, in contrast to the nonspecific duplex, its thermostability is high. Transition from the nonspecific to the zinc-specific duplex is observed at high zinc concentrations or at high temperatures. The transition is cooperative. These results are discussed in the context of the specific cation effects on the formation of intramolecular R·R·Y triplexes at $d(GA{\cdot}TC)_n$ DNA sequences.

DNA conformation is polymorphic. The degree of conformational polymorphism of DNA depends on both the base sequence and factors of the environment. In this respect, alternating $d(GA{\cdot}TC)_n$ sequences constitute an extreme case of highly polymorphic DNA. Depending on the environmental conditions, they can form a variety of different non-B-DNA conformations (Mirkin & Frank-Kamenetskii, 1994; Bernués & Azorín, 1995). At neutral pH in the presence of zinc or other transition metal ions, the $d(GA{\cdot}TC)_n$ sequences form intramolecular $d(GA{\cdot}GA{\cdot}TC)_n$ triplexes (Bernués et al., 1989; Beltrán et al., 1993). Increasing the metal-ion concentration results in a destabilization of the intramolecular triplex and formation of a $d(GA{\cdot}GA)_n$ hairpin (Beltrán et al., 1993; Martínez-Balbás & Azorín, 1993). To some extent, the conformational variability of $d(GA{\cdot}TC)_n$ sequences can be interpreted in terms of the conformational properties of their individual strands, especially of the purine strand. In a previous work, we have shown that alternating $d(GA)_n$ sequences are capable of forming both intramolecular foldbacks and bimolecular homoduplexes (Casasnovas et al., 1993). The unimolecular foldbacks are inevitably antiparallel and are stabilized by the formation of G·A pairs (Huertas et al., 1993). On the other hand, bimolecular complexes could either be parallel or be antiparallel. Environmental factors, in particular the type and concentration of counterions, dominantly contribute to the type of conformation adopted by the $d(GA)_n$ sequences. Formation of parallel-stranded $d(GA{\cdot}GA)_n$ homoduplexes has been described in the presence of magnesium (Rippe et al., 1992; Evertsz et al., 1994). However, magnesium ions do not induce the formation of intramolecular R·R·Y triplexes at $d(GA{\cdot}TC)_n$ sequences (Bernués et al., 1990). Other conformations have been proposed to be adopted by $d(GA)_n$ sequences under different experimental conditions (Lee et al., 1980; Antao et al., 1988; Lee, 1990). For example, an ordered single-stranded structure has been reported to be stabilized at acidic conditions (Dolinnaya & Fresco, 1992; Dolinnaya et al., 1993).

In this paper, we analyze the effect of divalent zinc cations on the structure of $d(GA)_n$ sequences. Using circular dichroism (CD)[1] spectroscopy and gel electrophoresis, we come to a conclusion that zinc can stabilize two types of duplex $d(GA)_n$ conformations. At low temperature, when the oligonucleotide is partially ordered, addition of zinc

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stabilizes a bimolecular complex, which is nonspecific with respect to cations present in solution since it is also detected in the presence of magnesium. However, if zinc is added under conditions when d(GA)$_n$ is exclusively single-stranded, formation of a different duplex is observed which is specific for zinc. These results may explain the different effects of zinc and magnesium on the formation of d(GA·GA·TC)$_n$ intramolecular triplexes.

MATERIALS AND METHODS

Oligonucleotides. All oligonucleotides were synthesized in an Applied Biosystems automatic synthesizer and purified by denaturing polyacrylamide gel electrophoresis.

Circular Dichroism Experiments. Circular dichroism spectra were measured on a Jasco dichrograph, Model J-720, and a Jobin-Yvon Mark IV dichrograph calibrated with isoandrosterone, in 1 cm path length cells placed in a thermostated holder. Oligonucleotide concentrations ranging between 0.05 and 0.09 mM (DNA phosphates) were determined on a Philips PU-8750 spectrophotometer using the molar extinction coefficient for the single-stranded form of d(GA)$_{20}$ of 11 050 M^{-1} cm^{-1} in the absorption maximum.

The spectra were recorded in either Tris-HCl (89 mM Tris, 40 mM HCl, pH 8.3) or Tris−borate (89 mM Tris, 89 mM boric acid, pH 8.3) buffer, in the presence of $ZnCl_2$ or $MgCl_2$ at the concentration indicated in each case. Some spectra were obtained in Tris−borate buffer at pH 6.9 obtained by the addition of concentrated HCl to Tris−borate, pH 8.3, buffer.

Polyacrylamide Gel Electrophoresis Analysis. For the electrophoretic analysis, d(GA)$_{20}$ was radioactively labeled with [γ-^{32}P]ATP and phage T4 polynucleotide kinase.

Gel electrophoresis experiments were carried out on 12% native polyacrylamide−Tris−borate (89 mM Tris, 89 mM boric acid, pH 8.3) gels containing $ZnCl_2$ or $MgCl_2$ at the concentration indicated in each case. Electrophoresis was allowed to proceed at 12 V cm^{-1} until the dye xylene cyanol had migrated around 12 cm. A large anodic reservoir (8 L) was used, so to reduce to a minimum the loss of the metals during the electrophoresis due to its deposition on the cathode. To maintain the temperature constant, electrophoresis was performed in a buffer-jacketed apparatus connected to a thermostated bath. Before electrophoresis, DNAs were incubated for 30 min under exactly the same buffer and temperature conditions at which the electrophoresis was to be carried out, in a final volume of 10 μL at a concentration of around 0.05 mM (DNA phosphates). After electrophoresis, gels were dried, and autoradiographs were recorded on Hyperfilm (Amersham). Densitometer scans were obtained in a Molecular Dynamics laser densitometer.

RESULTS

CD Spectra of d(GA)$_{20}$ in the Absence of Zinc. We have shown in a previous work (Casasnovas et al., 1993) that, under appropriate experimental conditions, alternating d(GA)$_{20}$ can form unimolecular foldbacks as well as bimolecular duplexes, the latter always being more abundant. Formation of these conformers was observed at low temperature (4 °C) at any pH in the range of 8.3−4.6. The thermal stability of these conformers is low so that, at 20 °C and pH 8.3, only the single-stranded form of d(GA)$_{20}$ can be detected by gel electrophoresis (Casasnovas et al., 1993). In agreement with these results, d(GA)$_{20}$ provides, at room temperature in Tris−borate buffer at neutral or alkaline pH, a CD spectrum corresponding to a single-stranded conformer (Figure 1), in which contributions from the constituent dinucleotides d(GA) and d(AG) are combined (Figure 1A, insert). The negative band at 285 nm originates from d(AG); then there is a shoulder in the CD spectrum of single-stranded d(GA)$_{20}$ that is followed by the positive band at 248 nm to which d(GA) mainly contributes. Also in line with our previous observations, temperature lowering stabilizes a conformer of d(GA)$_{20}$ whose CD spectrum is dominated by a strong positive band at around 267 nm (Figure 1). The stabilization of this conformer is more pronounced on lowering the pH to 6.9 (compare Figure 1A and Figure 1B). The arising CD spectrum mainly reflects the formation of the bimolecular duplex described above since, as indicated by gel electrophoresis (Casasnovas et al., 1993), this conformer dominates at the DNA concentrations used for the CD experiments. The conformational transition is evidently two-state but its cooperativity is low (Figure 1B, insert). The duplex is also stabilized by increasing ionic strength (not shown) and, remarkably, by chloride anion substitution for the borate anion in the buffer (Figure 1B, insert). The temperature-induced transition between the duplex and single strand of d(GA)$_{20}$ is quite cooperative in the Tris-HCl buffer, pH 8.3.

CD Spectra of d(GA)$_{20}$ in the Presence of Zinc. Addition of zinc shows different effects depending on whether it is added to single-stranded d(GA)$_{20}$ or to its duplex form. Addition of zinc to single-stranded d(GA)$_{20}$ (e.g., at room temperature in Tris-HCl or Tris−borate, pH 8.3) transforms the oligonucleotide into a novel conformation which is neither single-stranded nor the duplex described above. Its CD spectrum contains a three bump positive band in the long-wavelength region and a strong negative band at 252 nm (Figure 2A). This type of CD spectrum, essentially consisting of a positive long-wavelength band and a negative short-wavelength band, is characteristic for B-DNA conformations of poly[d(GC)] or poly[d(AT)] (Guschlbauer, 1988).

When the effect of zinc on the dinucleotides d(GA) and d(AG) was studied, no changes in their CD spectra were observed up to Zn/P = 10 (not shown). Higher zinc concentrations induced scattering caused by dinucleotide aggregation. The scattering was still enhanced at higher temperatures, but the CD spectra shapes did not change with any of the dinucleotides. Therefore, the zinc-induced changes in the CD spectra of d(GA)$_{20}$ originate from conformational alterations of the oligonucleotide.

The zinc-induced structural transition of d(GA)$_{20}$ takes place within Zn/P = 3−8 (Figure 2A, insert). More than two strict conformers are involved in this transition because the CD spectra do not intersect in isoelliptic points. The resulting zinc-stabilized conformer is very thermostable. It melts cooperatively at around 70 °C (Figure 2B, insert), and the melting is irreversible (not shown). Electrophoretic experiments described below (Figures 6 and 7) indicate that this zinc-induced conformation is a duplex.

Remarkably, the effects are different when the metal ion is added under conditions at which d(GA)$_{20}$ coexists in the duplex and single-stranded forms (e.g., at low temperature

[1] Abbreviations: bp, base pair(s); CD, circular dichroism; *M*, molecular mass; R, purine residue; Y, pyrimidine residue.

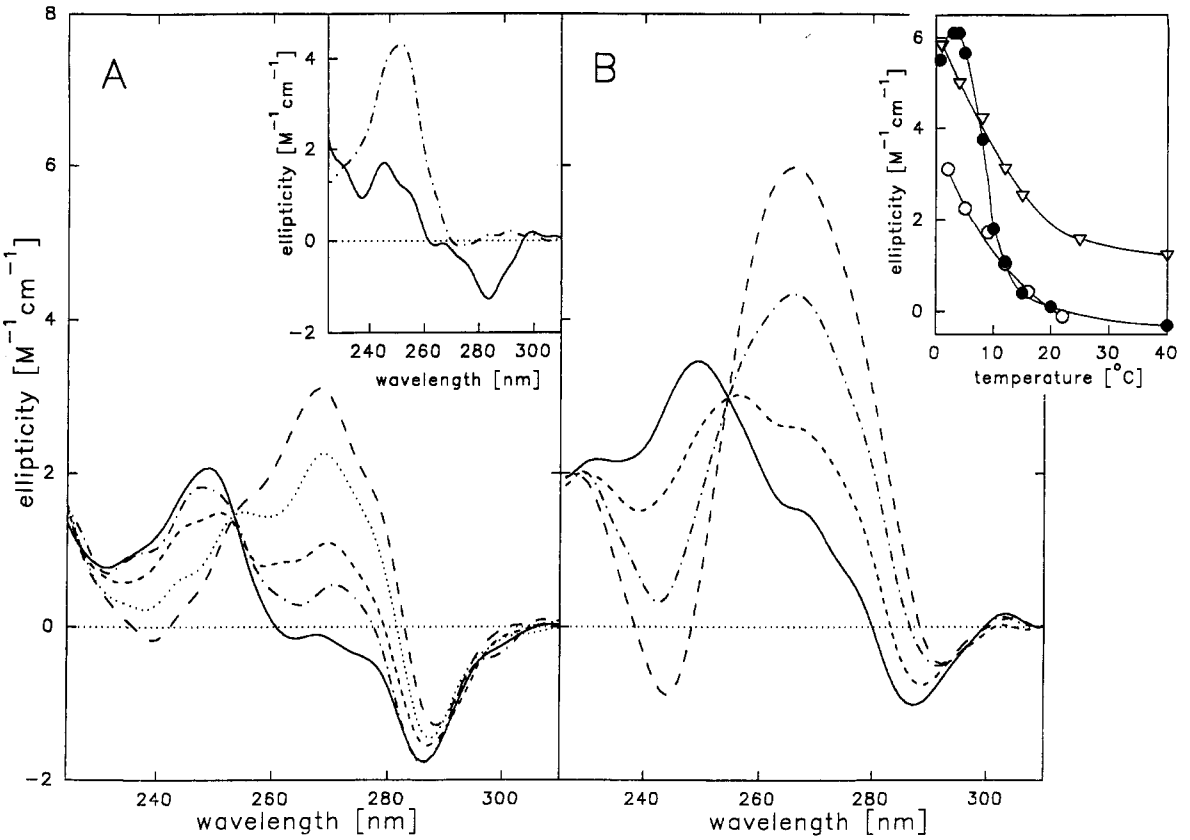

FIGURE 1: CD spectra of $d(GA)_{20}$ measured in Tris−borate buffer, pH 8.35 (panel A) and 6.94 (panel B) at the following temperatures: (panel A) (—) 22, (— • —) 16, (- - -) 12, (•••) 5, and (— —) 2 °C; (panel B) (—) 25, (- - -) 15, (— • —) 8, and (— —) 1 °C. The spectra reflect the isomerization of $d(GA)_{20}$ between its single-stranded and double-stranded forms. Insert A: CD spectra of the dinucleotides (—) d(ApG) and (— • —) d(GpA) measured in Tris−borate, pH 8.35 at 20 °C. Insert B: Ellipticity (267 nm) dependence of $d(GA)_{20}$ on temperature in Tris−borate, pH 8.35 (○), Tris−borate, pH 6.94 (▽), and Tris-HCl, pH 8.35 (●).

in Tris−borate buffer, pH 8.3; see Figure 1). In this case, addition of zinc up to Zn/P = 6 (Figure 3A) provides a CD spectrum which is very similar to that arising at low temperature in the absence of zinc, but its amplitudes are much higher (compare Figures 1 and 3A). As shown below, the same CD spectrum is also observed in the presence of Mg^{2+} (Figure 5), indicating that this effect is nonspecific. This nonspecific conformer arises cooperatively through a two-state transition from the single strand, and its thermostability is low. The melting temperature is about 30 °C. Above the melting temperature, the CD spectrum of $d(GA)_{20}$ bears no traces of the presence of zinc which thus had to dissociate from the oligonucleotide. Melting of this nonspecific conformer is reversible (not shown). Further addition of zinc above Zn/P = 6 elicits a two-state transition of $d(GA)_{20}$ from the nonspecific into the zinc-specific conformer (Figure 3B). The CD spectra obtained at high Zn/P are similar to those obtained when zinc was added to single-stranded $d(GA)_{20}$ (compare Figures 2A and 3B). Under these conditions, the nonspecific and zinc-specific conformers of $d(GA)_{20}$ coexist as reflected by the presence of the maximum at around 268 nm even at high Zn/P = 10.

Transition from the nonspecific to the zinc-specific conformer of $d(GA)_{20}$ is also promoted by increasing temperature. Figure 4 shows the CD spectra of $d(GA)_{20}$ obtained in the presence of Zn/P = 8.6 at increasing temperature. At low temperature, the nonspecific conformer is still present as reflected by the maximum at 268 nm. Increasing temperature results in the stabilization of the zinc-specific conformer which, at 30 °C, predominates as indicated by the CD spectrum recorded at this temperature. Unlike at lower zinc concentrations, increasing temperature does not dissociate zinc from the oligonucleotide but rather shifts the equilibrium toward the formation of the zinc-specific conformer which cooperatively denatures only above 70 °C (Figure 4). The insert of Figure 4 shows these two consecutive processes, i.e., the stabilization of the zinc-specific conformer between 0 and 30−40 °C, and its melting above 70 °C. The denaturation is irreversible and is accompanied by light scattering due to oligonucleotide aggregation which is likely to stand behind the irreversibility of the melting of the zinc-specific conformer.

Similar results were obtained when the metal ion was added in the same buffer but at pH 6.9. Under these conditions, addition of zinc stabilizes the nonspecific conformer, and the CD spectra characteristic of the zinc-specific conformer do not appear unless the temperature is increased above 40 °C (not shown).

Cation Specificity of the Zinc-Specific Conformer. Parallel experiments with divalent magnesium cations were performed in order to assess the specificity of the zinc-specific conformer. Magnesium was added to $d(GA)_{20}$ under optimum conditions for the formation of the specific conformer, at room temperature in Tris-HCl, pH 8.3 (see Figure 2). The CD spectrum dependence of $d(GA)_{20}$ on the concentration of $MgCl_2$ (Figure 5) demonstrates that the nonspecific conformer is only formed. The zinc-specific conformer appears neither at high $MgCl_2$ concentrations (Figure 5, insert A) nor at increased temperatures (Figure 5, insert B). The increasing temperature only denatures the nonspecific duplex

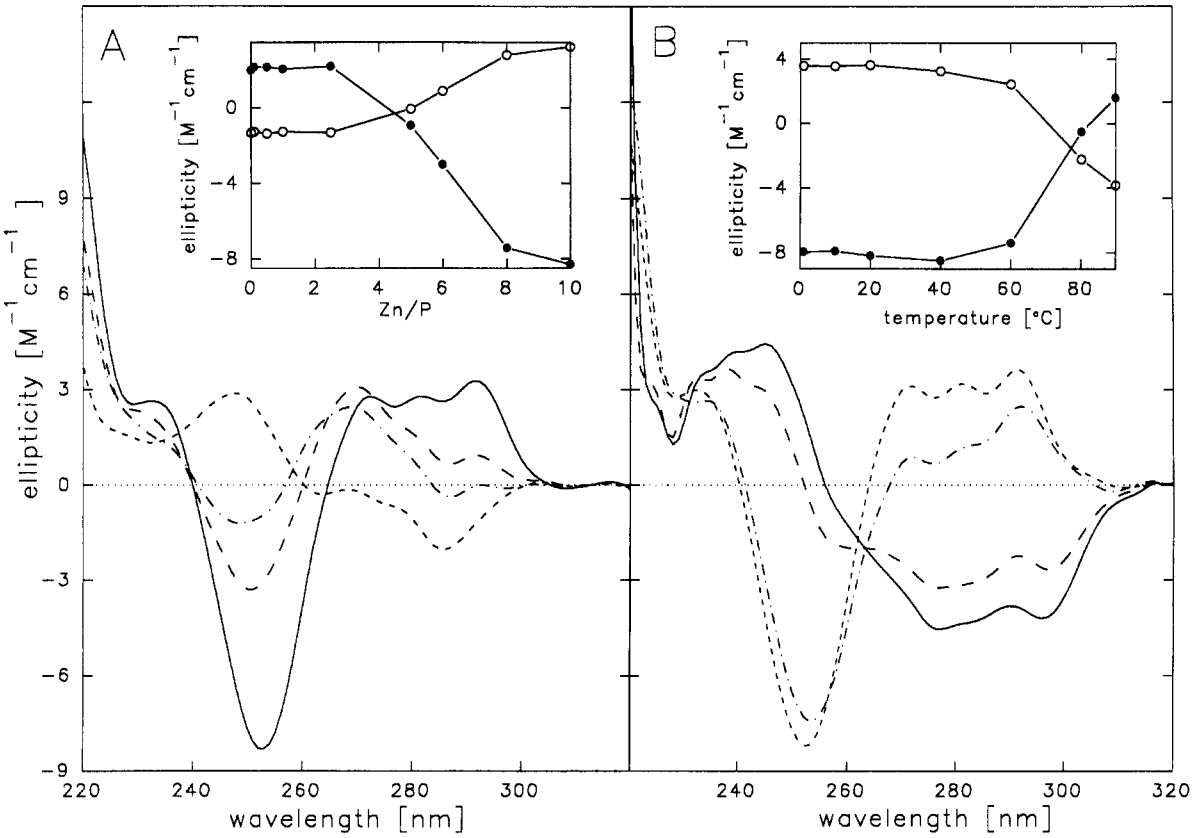

FIGURE 2: CD spectra of d(GA)$_{20}$ reflecting the formation of the zinc-specific duplex. The spectra were measured in Tris-HCl buffer, pH 8.3. Panel A: 25 °C, Zn/P ratios of (- - -) 0, (— • —) 5, (— —) 6, and (—) 10. Insert: Formation of the zinc-specific duplex monitored by ellipticity changes at 253 (●) and 291 (○) nm. Panel B: CD spectra of d(GA)$_{20}$ under conditions of panel A containing 10 Zn/P measured at (- - -) 20, (— • —) 60, (— —) 80, and (—) 90 °C. Insert: Thermal melting of the zinc-specific duplex of d(GA)$_{20}$ monitored as in the insert of panel A.

(Figure 5, insert B), while the denaturation is completely reversible like in the case of the nonspecific conformer stabilized by zinc. The set of CD spectra recorded during the temperature-induced denaturation is identical to that shown in Figure 5, recorded at constant temperature and different $MgCl_2$ concentrations. The spectra thus reflect the oligonucleotide denaturation caused by magnesium dissociation.

Electrophoretic Analysis of the Zinc-Specific Conformer. The electrophoretic behavior of d(GA)$_{20}$ in the presence and absence of zinc is in line with the conclusions derived from the CD measurements. Figure 6 shows the effect of increasing zinc concentration on the electrophoretic behavior of d(GA)$_{20}$ in Tris—borate buffer, pH 8.3, at room temperature. These are the conditions which, according to the CD results, promote the formation of the zinc-specific conformer. In line with the CD data (Figure 1A), the electrophoretic migration of d(GA)$_{20}$ observed in the absence of zinc indicates that, under these experimental conditions, the oligonucleotide is mainly single-stranded (Figure 6, lane 0). Addition of zinc up to a Zn/P = 3 shows no effect upon the electrophoretic behavior of d(GA)$_{20}$ (Figure 6, lanes 1 and 2). However, important electrophoretic changes are observed upon increasing further the zinc concentration (Figure 6, lanes 3—5). These results are in general agreement with the CD measurements which show formation of the zinc-specific conformer only above Zn/P = 3 (Figure 2A, insert). At Zn/P = 7, formation of an electrophoretic species migrating like a bimolecular duplex is detected (Figure 6, lane 3). At this Zn/P ratio, a significant amount of single-stranded d(GA)$_{20}$ is still observed. Further increasing the Zn/P ratio results in disappearance of the single-strand which is no longer detected at Zn/P = 16 (Figure 6, lane 5). At this Zn/P ratio, in addition to the bimolecular duplex observed at Zn/P = 7, several other electrophoretic species are detected migrating faster than the bimolecular duplex. These additional fast migrating species, which are already observed at Zn/P = 10 (Figure 6, lane 4), are also bimolecular as they have a slower electrophoretic migration than the single-stranded form. Furthermore, the CD results clearly show an absence of single strands at these high Zn/P ratios (Figure 2A). Most likely, these fast migrating species correspond to different types of bimolecular duplexes differing by the presence of intramolecular foldbacks (see Figure 9). These results indicate that the zinc-specific conformer is not a single electrophoretic species, which is in agreement with the CD results indicating that more than two conformers are involved in the transition (Figure 2).

Electrophoretic Analysis of the Nonspecific Conformer. Similar to the above CD analysis, the electrophoretic results are different when the divalent zinc cations are added to d(GA)$_{20}$ under conditions promoting formation of the nonspecific conformer, i.e., at low temperature in Tris—borate, pH 8.3 (Figure 7A). Under these conditions, d(GA)$_{20}$ is mostly single-stranded, but small ingredients of both intramolecular and bimolecular complexes are also detected (Figure 7A, lane 0). This is in agreement with the CD results shown in Figure 1A. Addition of zinc, even at low Zn/P = 1, generates a well-defined electrophoretic species migrating as a bimolecular complex showing an apparent molecular

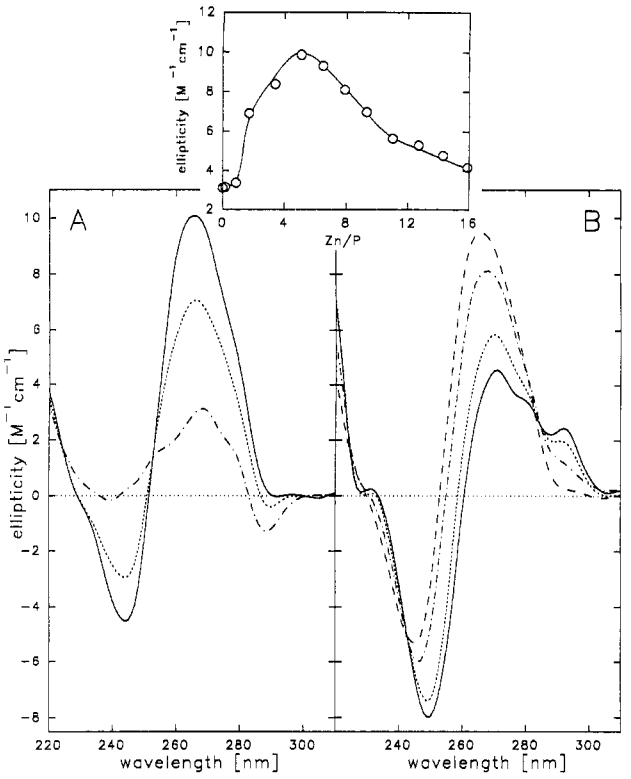

FIGURE 3: CD spectra of d(GA)$_{20}$ reflecting its interaction with zinc. The spectra were measured in Tris−borate, pH 8.35 at 2 °C. Panel A: Zn/P ratio of (− • −) 0, (•••) 1.6, and (—) 4.8 (stabilization of the nonspecific conformer). Panel B: Zn/P ratio of (− −) 6.4, (− • −) 7.9, (•••) 11.1, and (—) 15.9 (stabilization of the zinc-specific conformer). Insert: zinc-induced changes in the ellipticity of d(GA)$_{20}$ at 268 nm.

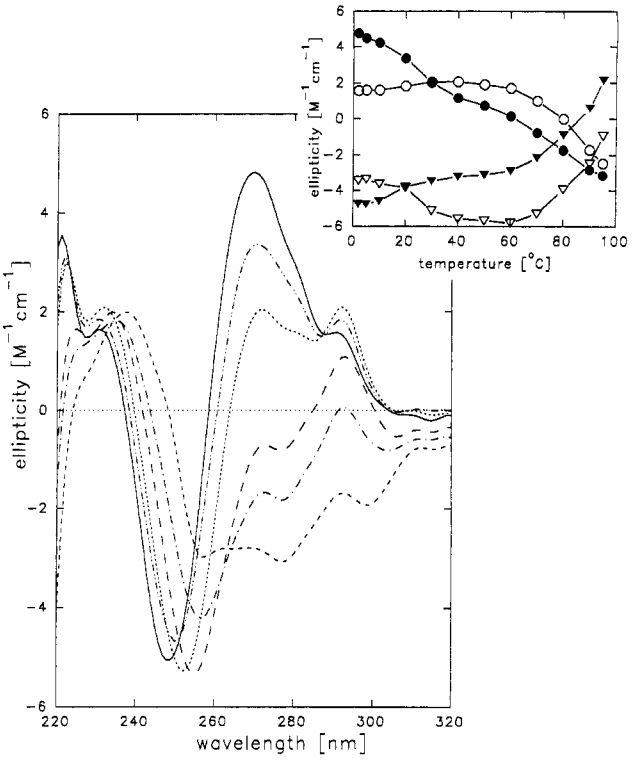

FIGURE 4: Temperature dependence of the CD spectra of d(GA)$_{20}$ in Tris−borate, pH 8.35, to which 8.6 Zn/P was added at 1 °C. The spectra were measured at the following temperatures: (—) 2, (− • • −) 20, (•••) 30 (stabilization of the zinc-specific conformer), (− −) 70, (− • −) 80, and (- - -) 90 °C (denaturation). Insert: temperature-induced changes in the CD spectra shown in the figure monitored by the ellipticity at (○) 291, (●) 271, (▼) 246, and (▽) 254 nm.

weight (M) of around 42.7 bp (Figure 7A, lane 1). Increasing the Zn/P to 3 stabilizes this bimolecular complex (Figure 7A, lane 2), which, according to the CD experiments, must correspond to the nonspecific conformer (Figure 3A). In accordance with this interpretation, the same electrophoretic species is observed when the electrophoresis is carried out in the presence of magnesium at either 4 °C or 23 °C (Figure 7C).

Increasing further the zinc concentration results in the formation of new electrophoretic species (Figure 7A, lanes 3−5). Notably, at Zn/P = 7−10, a second electrophoretic species migrating as a bimolecular complex is detected (Figure 7A, lanes 3 and 4, and Figure 7B), most likely reflecting formation of the zinc-specific conformer of d(GA)$_{20}$ which, according to the CD results shown in Figure 3B, coexists with the zinc-stabilized nonspecific duplex under these conditions. As shown by the densitometer scans in Figure 7B, this second bimolecular complex shows an electrophoretic mobility (apparent M = 43.6 bp) lower than that corresponding to the nonspecific duplex. These two bimolecular species are also observed at high Zn/P = 16, but in this case, a few discrete bands migrating faster are also detected (Figure 7A, lane 5). This electrophoretic pattern is similar to that obtained when zinc is added at room temperature (Figure 6). These results are in accordance with CD experiments revealing a similar transition to the zinc-specific conformer at low temperature and high Zn/P (Figure 3B). The electrophoretic migration of the zinc-specific conformer at Zn/P = 7−10 demonstrates its bimolecular nature.

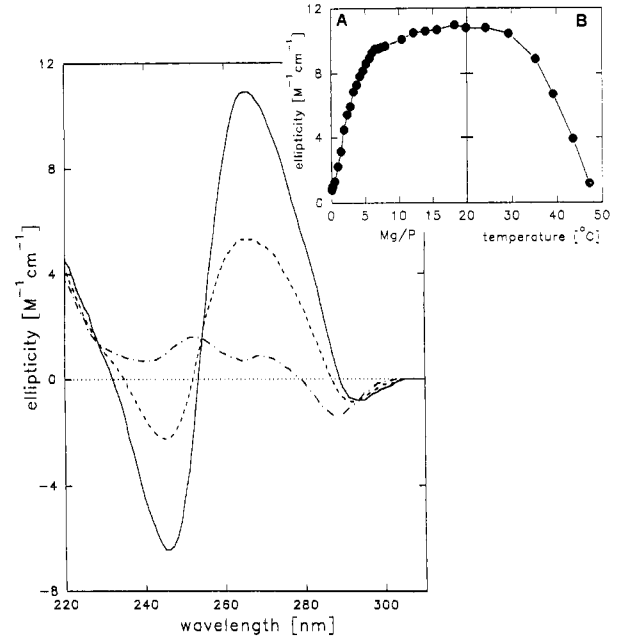

FIGURE 5: CD spectra of d(GA)$_{20}$ reflecting its interaction with magnesium. The spectra were measured in Tris-HCl, pH 8.35 at 25 °C. Mg/P: (− • −) 0, (− −) 2.4, and (—) 8. Insert: The magnesium-induced stabilization of the nonspecific conformer of d(GA)$_{20}$ (panel A) and its destabilization by temperature (panel B), monitored by the changes in ellipticity at 268 nm.

As inferred from the CD spectra (Figure 4), the transition from the nonspecific duplex to the zinc-specific conformer is also promoted by increasing temperature. A similar

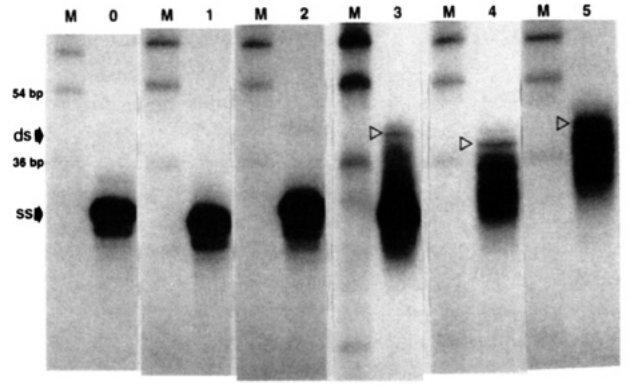

FIGURE 6: Polyacrylamide gel electrophoretic analysis of the zinc-specific conformer of d(GA)$_{20}$. Electrophoresis was carried out at 20 °C at increasing Zn/P: 0 (lane 0), 1 (lane 1), 3 (lane 2), 7 (lane 3), 10 (lane 4), and 16 (lane 5). Lanes M correspond to oligomers of an 18-mer oligonucleotide used as molecular weight standards. The positions of the dimer and trimer are indicated on the left. The electrophoretic migration corresponding to the single-stranded (ss) and double-stranded (ds) forms of d(GA)$_{20}$ is also indicated on the left. Empty arrowheads indicate the formation of double-stranded forms.

transition to the zinc-specific conformer was observed when the electrophoretic behavior of the nonspecific duplex, obtained at 4 °C and Zn/P = 10, was determined at increasing temperature (Figure 8). The same two very close migrating electrophoretic species observed at 4 °C are also observed when the electrophoresis is carried out at 12 °C (Figure 8, lane 1). However, when the electrophoresis were carried out at 20 or 30 °C, formation of a discrete set of bands was observed (Figure 8, lanes 2 and 3). These electrophoretic patterns are very similar to those obtained when zinc was added at room temperature (Figure 6). Interestingly, at 30 °C, the formation of electrophoretic species of higher apparent molecular weight, ranging from about 70 to 90 bp, is detected (Figure 8, lane 3). These high molecular weight species must be multistranded complexes.

DISCUSSION

Alternating d(GA)$_n$ DNA sequences are highly polymorphic. Depending on the precise environmental conditions, they can adopt a variety of different structural conformations, including double-stranded and tetrastranded forms (Lee et al., 1980; Antao et al., 1988; Lee, 1990; Dolinnaya & Fresco, 1992; Rippe et al., 1992; Casasnovas et al., 1993; Dolinnaya et al., 1993; Huertas et al., 1993; Evertsz et al., 1994). Counterions are important structural determinants of d(GA)$_n$ DNA sequences. In this paper, we have studied in detail the effects of divalent zinc cations on the conformation of a d(GA)$_{20}$ oligonucleotide. Figure 9 summarizes the results. As reported earlier (Casasnovas et al., 1993), d(GA)$_{20}$ forms both unimolecular foldback structures and bimolecular duplexes at low temperature in the absence of zinc. The CD spectra of d(GA)$_{20}$ obtained under these conditions (Figure 1) also suggest formation of these duplexes. Though the unimolecular foldbacks and bimolecular duplexes coexist under a wide range of pH conditions, the latter are always more abundant. In particular, at pH 7 and at the DNA concentrations used for the CD experiments, the bimolecular form is the only species that can be detected by gel electrophoresis (Casasnovas et al., 1993). Therefore, the CD spectra shown in Figure 1 principally reflect formation of

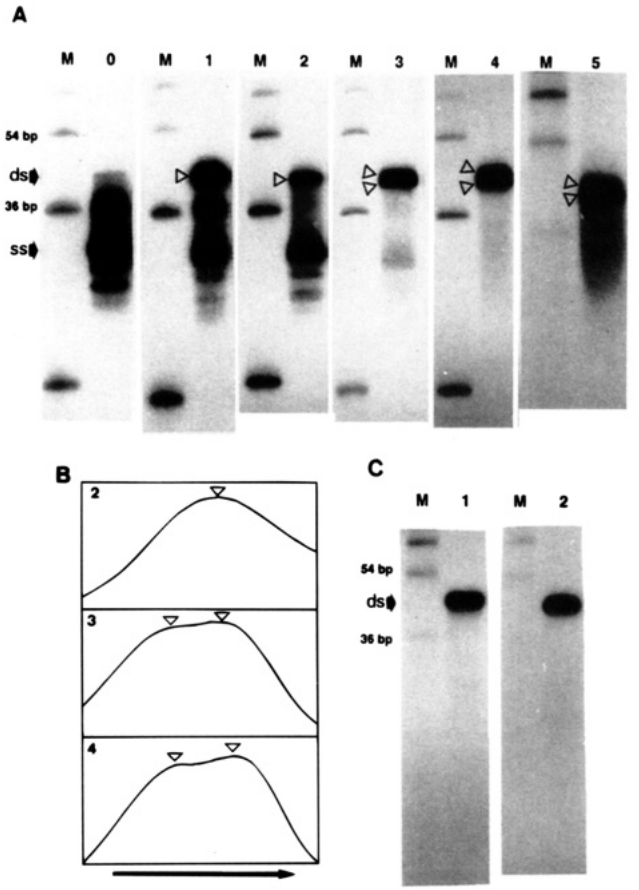

FIGURE 7: Polyacrylamide gel electrophoretic analysis of the nonspecific conformer of d(GA)$_{20}$. Panel A: Electrophoresis was performed at 4 °C at increasing Zn/P: 0 (lane 0), 1 (lane 1), 3 (lane 2), 7 (lane 3), 10 (lane 4), and 16 (lane 5). Lanes M correspond to oligomers of an 18-mer oligonucleotide used as molecular weight standards. The positions of the dimer and trimer are indicated on the left. The electrophoretic migration corresponding to the single-stranded (ss) and double-stranded (ds) forms of d(GA)$_{20}$ is also indicated on the left. Empty arrowheads indicate the formation of double-stranded forms. Panel B: Densitometer scans of lanes 2 (top), 3 (middle), and 4 (bottom) of panel A. The arrow indicates the direction of electrophoresis. Only the regions corresponding to the bimolecular complexes are presented. Two different duplexes of very similar electrophoretic migration are detected in the scans of lanes 3 and 4. Panel C: Electrophoresis was carried out at 4 °C (lane 1) and 23 °C (lane 2) in the presence of magnesium at a Mg/P = 16.

the bimolecular complexes. These bimolecular forms are stabilized by low temperature, increasing ionic strength, or lowering pH.

The effect of the divalent zinc cations on the conformation of d(GA)$_{20}$ depends on the experimental conditions at which they are added. At neutral pH and low temperature, zinc stabilizes the bimolecular duplex existing (though not exclusively) under these conditions. The same bimolecular duplex is also stabilized by magnesium. The electrophoretic studies clearly demonstrate the bimolecular nature of this structural conformer. In contrast, a zinc-specific conformer is detected if zinc is added to d(GA)$_{20}$ under conditions distant from those stabilizing the nonspecific bimolecular duplex, for example, at room temperature and pH 8.3. This zinc-specific conformation includes more than a single strict conformer. A transition from the nonspecific duplex to the zinc-specific conformer is observed either at high Zn/P and low temperature (Figure 3) or at increased temperature and moderate Zn/P (Figure 4). The low thermostability, revers-

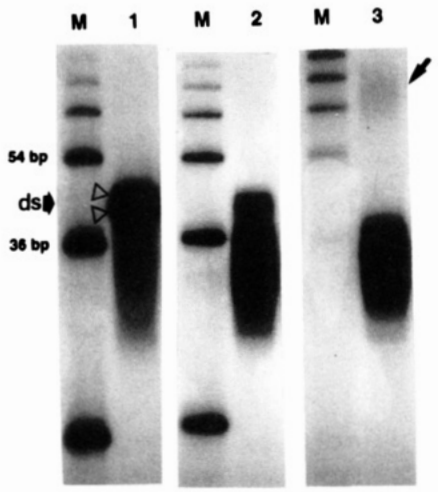

FIGURE 8: Gel electrophoretic analysis of the temperature-induced transition from the nonspecific to the zinc-specific conformer of $d(GA)_{20}$. The oligonucleotides were incubated at 4 °C and Zn/P = 10 in Tris−borate, pH 8.3. Electrophoresis was carried out at 12 °C (lane 1), 20 °C (lane 2), and 30 °C (lane 3). The arrow in lane 3 indicates the formation of complexes of slow electrophoretic mobility. Lanes M correspond to oligomers of an 18-mer oligonucleotide used as molecular weight standards. The positions of the dimer and trimer are indicated on the left. The electrophoretic migration corresponding to the double-stranded (ds) forms of $d(GA)_{20}$ is also indicated on the left.

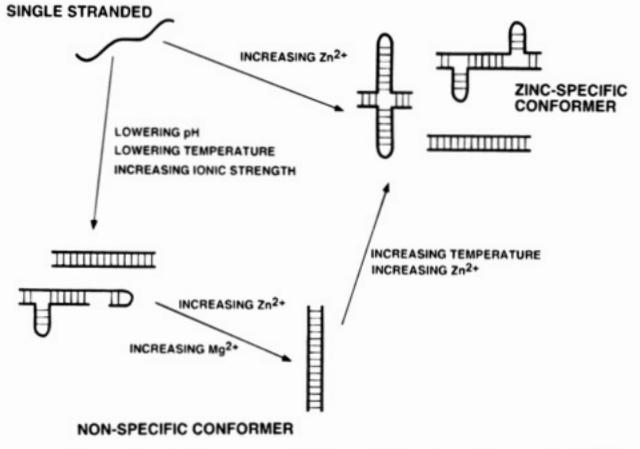

FIGURE 9: Schematic representation of the transitions studied in this paper. Single-stranded $d(GA)_{20}$ undergoes transition to a bimolecular complex upon lowering the pH, lowering the temperature, or increasing the ionic strength. Zinc added under these conditions stabilizes this nonspecific duplex which is also observed in the presence of magnesium. Zinc added under conditions where $d(GA)_{20}$ is exclusively single-stranded induces transition to the zinc-specific conformations. Transition of the nonspecific to the zinc-specific conformer is promoted by either high Zn/P at low temperature or by increasing temperature at moderate Zn/P.

ibility, and facile dissociation of the stabilizing divalent cations belong among properties discriminating the nonspecific duplex from the zinc-specific conformer. Thus, zinc binds to the oligonucleotide by two qualitatively different mechanisms. The first is nonspecific and probably involves electrostatic interactions with the backbone phosphate groups. In this case, zinc operates like monovalent cations or divalent magnesium cations. The second mechanism requires higher zinc concentrations and presumably involves coordination to the bases. It is known that zinc, as well as most transition metal ions, is capable of binding to the DNA bases (Saenger, 1984). The preferred site for metal ion coordination to the DNA bases is the N7 group of the purine residues, principally of guanines (Saenger, 1984). Therefore, it is likely that the zinc-specific conformer of $d(GA)_{20}$ arises from the specific coordination of the metal to the bases. As mentioned above, the zinc-specific conformer is stabilized by increasing temperature, suggesting also that metal ion coordination is involved in its formation.

The CD spectra obtained at low temperature in the presence of zinc (Figure 3A) or magnesium (Figure 5), corresponding to the nonspecific duplex of $d(GA)_{20}$, are very similar to those reported earlier for parallel-stranded $d(GA)_n$ homoduplexes (Rippe et al., 1992), strongly suggesting that the metal-induced non-specific duplex described here is parallel-stranded. Similar CD spectra are also obtained in the absence of any added metal ion (Figure 1). However, the ellipticity values of the maximum occurring at around 268 nm are always higher in the presence of metals (compare Figure 1 with Figures 3A and 5), indicating that, in the absence of metals, several related conformers might coexist.

On the other hand, the zinc-specific conformation of $d(GA)_{20}$ shows a radically different CD spectrum (Figure 2A). From the electrophoretic results shown in Figures 6 and 7, it follows that the zinc-specific conformer of $d(GA)_{20}$ does not correspond to a single molecular species. This interpretation is in agreement with the CD results. However, at moderate zinc concentration, the zinc-specific conformer principally corresponds to a bimolecular complex (Figure 6, lane 3) which, at low temperature, coexists with the nonspecific duplex (Figure 7A,B, lanes 3 and 4). The two duplexes show very similar though not identical electrophoretic mobilities. It has been reported that the electrophoretic mobility of DNA duplexes depends on the strand orientation. In general, parallel-stranded duplexes migrate faster than equivalent antiparallel-stranded ones (Germann et al., 1988; Ramsing & Jovin, 1988; van de Sande et al., 1988; Rippe et al., 1989; Evertsz et al., 1994). Therefore, the different electrophoretic mobilities of the nonspecific and the zinc-specific duplexes may reflect a difference in strand polarity. Consistent with this interpretation, the nonspecific duplex, which according to the CD results is likely to be parallel-stranded, shows a faster electrophoretic migration than the zinc-specific duplex. Under conditions far from denaturation, the change in strand polarity should be especially difficult. Thus, it is imaginable that, if the transition from the nonspecific to the zinc-specific duplex involves a change in strand orientation, it will give rise to metastable kinetic intermediates containing three or four strands. Such species can be traced in Figure 8. Altogether, these results provide circumstantial evidence in favor of an antiparallel orientation of the zinc-specific duplex. Further experiments would be required before unequivocally determining the strand polarity of the zinc-specific duplex. Increasing further the zinc concentration results in the appearance of several electrophoretic species which we interpret as reflecting the formation of duplexes containing various foldbacks of different length (Figure 9).

The present results are relevant to the different effects of magnesium and zinc on the stability of the $d(GA{\cdot}GA{\cdot}TC)_n$ intramolecular triplexes. It follows from the results reported here and elsewhere (Rippe et al., 1992) that $d(GA)_n$ sequences have a high tendency to form parallel-stranded homoduplexes, which interferes with the intramolecular $d(GA{\cdot}GA{\cdot}TC)_n$ triplex formation where the homopurine strands are antiparallel. Magnesium stabilizes both the

parallel-stranded d(GA)$_n$ homoduplex and the regular double-stranded form of d(GA·TC)$_n$ as well. This may explain why the d(GA·GA·TC)$_n$ intramolecular triplex is unstable in the presence of magnesium. On the other hand, under appropriate conditions, zinc destabilizes the parallel-stranded d(GA)$_n$ homoduplex and promotes formation of a zinc-specific duplex which is likely to be antiparallel-stranded. Moreover, zinc is also known to destabilize the regular double-stranded B-DNA (Saenger, 1984). These zinc-induced effects are likely to facilitate formation of the d(GA·GA·TC)$_n$ intramolecular triplexes and, in particular, of the d(GA·GA)$_n$ intramolecular hairpins, in which the orientation of the purine strands is antiparallel.

Alternating d(GA·TC)$_n$ DNA sequences are quite abundant in eukaryotic genomic DNA (Manor et al., 1988). Several lines of evidence suggest that these simple repeating sequences are involved in processes of DNA recombination which are likely the consequence of their peculiar structural properties (Sekiya et al., 1981; Hentschel, 1982; Glikin et al., 1983; Mason et al., 1983; Richards et al., 1983; Hunt et al., 1984; Collier et al., 1988; Weinreb et al., 1990; Bernués et al., 1991). Here we have shown that divalent cations have important and specific effects on the conformation of d(GA)$_n$ sequences. It is also of interest that zinc cations stabilize a cooperatively melting foldback conformation of a d(TC)$_{20}$ oligonucleotide (unpublished results). This suggests that physiological concentrations of divalent cations might unfold the d(GA·TC)$_n$ sequences modulating the equilibria between their different structural conformers.

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