Conformational Transitions of Alternating Purine-Pyrimidine DNAs in Perchlorate Ethanol Solutions

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ABSTRACT Conformational transitions of poly(dA-dC) · poly(dG-dT), poly(dA-dT) · poly(dA-dT), and other alternating purine-pyrimidine DNAs were studied in aqueous ethanol solutions containing molar concentrations of sodium perchlorate, which is a novel solvent stabilizing non-B duplexes of DNA. Using CD and UV absorption spectroscopies, we show that this solvent unstacks bases and unwinds the B-forms of the DNAs to transform them into the A-form or Z-form. In the absence of divalent cations poly(dA-dC) · poly(dG-dT) can adopt both of these conformations. Its transition into the Z-form is induced at higher salt and lower ethanol concentrations, and at higher temperatures than the transition into the A-form. Submillimolar concentrations of $NiCl_2$ induce a highly cooperative and slow A-Z transition or Z-Z′ transition, which is fast and displays low cooperativity. Poly(dA-dT) · poly(dA-dT) easily isomerizes into the A-form in perchlorate-ethanol solutions, whereas high perchlorate concentrations denature the polynucleotide, which then cannot adopt the Z-form. At low temperatures, however, $NiCl_2$ also cooperatively induces the Z′-form in poly(dA-dT) · poly(dA-dT). Poly(dI-dC) · poly(dI-dC) is known to adopt an unusual B-form in low-salt aqueous solution, which is transformed into a standard B-form by the combination of perchlorate and ethanol. $NiCl_2$ then transforms poly(dI-dC) · poly(dI-dC) into the Z′-form, which is also adopted by poly(dI-br^{5}dC) · poly(dI-br^{5}dC).

INTRODUCTION

Biological properties are, to some extent, controlled by conformational properties of the genomic DNA. Simple sequence repeats, e.g., alternating $(dA\text{-}dT)_n \cdot (dA\text{-}dT)_n$ or $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$, confer an extreme conformational polymorphism on the DNA double helix (Vorlíčková and Kypr, 1985). The $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequences have recently been demonstrated to associate into four-stranded complexes (Gaillard and Strauss, 1994). The simple sequence repeats (microsatellites) are very abundant in eukaryotic genomes (Vogt, 1990; Sarkar et al., 1991). The genomic locations of the $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ repeats are remarkably conserved between, for example, humans and pigs (Wintero et al., 1992) or humans and chimpanzees (Deka et al., 1994). Instability of the $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ microsatellites is an early genetic event in a number of human cancers (Rhyu et al., 1994; Patel et al., 1994; Zenklusen et al., 1994; Uchida et al., 1995).

Linear unconstrained DNAs mostly adopt variants of the B-form in low and moderate salt aqueous solutions, but there are solvents or agents that promote their non-B conformers. The well-known solvents that destabilize B-DNA and stabilize non-B conformations include high-alcohol and very low salt concentrations (Ivanov et al., 1974), high-salt concentrations in aqueous solution (Pohl and Jovin, 1972; Vorlíčková et al., 1983), trivalent and tetravalent cations (Bloomfield, 1991), low divalent cation concentrations in low-salt aqueous solutions (Behe and Felsenfeld, 1981; Vorlíčková at al., 1988; Bernues et al., 1990), and high monovalent plus low $NiCl_2$ concentrations (Bourtayre et al., 1987). Here we analyze still another solvent that stabilizes non-B conformers which contains relatively low ethanol and molar sodium perchlorate concentrations.

This solvent was first reported (Jovin et al., 1983) to induce the Z-form in poly(dA-dC) · poly(dG-dT). However, Riazance-Lawrence and Johnson (1992) suggested that it was traces of divalent cations that stabilized the Z-form. They demonstrated that a careful removal of divalent cations not only prevented poly(dA-dC) · poly(dG-dT) from adopting the Z-form, but the polynucleotide transformed into A-form instead. This inspired us to study conformational properties of poly(dA-dC) · poly(dG-dT) and other alternating purine-pyrimidine DNAs when exposed to the combination of perchlorate and ethanol, to map conditions inducing transitions among their various duplex conformations. Here we report results of this extensive work and compare the conformational behavior of poly(dA-dC) · poly(dG-dT) and a number of other alternating purine-pyrimidine DNAs.

MATERIALS AND METHODS

Poly(dA-dC) · poly(dG-dT) was from Boehringer, and poly(dA-dT) · poly(dA-dT) and poly(dI-dC) · poly(dI-dC) were produced by Pharmacia. Poly(dI-br^{5}dC) · poly(dI-br^{5}dC) (Vorlíčková and Sági, 1991) and poly(dA-br^{5}dC) · poly(dG-dT) (Mirau et al., 1986) were kindly provided by J. Sági and T. Jovin, respectively. Unless stated otherwise, the polynucleotides were dissolved in 10 mM Tris-HCl, 0.1 mM EDTA, pH 7.5. Solid $NaClO_4$ and 96% ethanol were added to the polynucleotide solutions to get their desired concentrations. The polynucleotides were purified as described in the literature (Riazance-Lawrence and Johnson, 1992).

UV absorption and CD spectra were measured, respectively, on a Philips PU 8750 spectrophotometer and a Jobin-Yvon Mark IV dichrograph in 1-cm or 0.5-cm path length cells placed in a thermostated holder.

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Polynucleotide concentrations (0.08–0.25 mM in DNA phosphates) were determined by using molar extinction coefficients, in their UV absorption maxima, of 6640 $M^{-1}cm^{-1}$ for poly(dA-dT) · poly(dA-dT) (Sági and Ötvös, 1980), 6500 $M^{-1}cm^{-1}$ for poly(dA-dC) · poly(dG-dT) (Wells et al., 1970), 6200 $M^{-1}cm^{-1}$ for poly(dA-br^{5}dC) · poly(dG-dT) (Mirau et al., 1986), 5900 and 4600 $M^{-1}cm^{-1}$ for poly(dI-dC) · poly(dI-dC) and poly(dI-br^{5}dC) · poly(dI-br^{5}dC), respectively (Vorlíčková and Sági, 1991). The dichrograph was calibrated with isoandrosterone.

RESULTS

B-A transition of poly(dA-dC) · poly(dG-dT)

Poly(dA-dC) · poly(dG-dT) provides the standard weak conservative CD spectrum in low-salt aqueous solution (Gray and Ratliff, 1975). Addition of sodium perchlorate gradually alters the CD spectrum to a nonstandard shape containing two separate positive bands located at 263 nm and 285 nm, and a relatively deep negative band at 212 nm (Fig. 1). At low temperature and a constant 3 M sodium perchlorate, the polynucleotide cooperatively transforms into the A-DNA conformation within 20% and 30% ethanol (Fig. 1, *inset A*). The A-DNA gives a strong positive CD band at 263 nm and a negative band at 212 nm (Fig. 1), like the A-DNA of this polynucleotide induced by high ethanol and very low salt concentrations (Gray and Ratliff, 1975), or the molar sodium perchlorate and low ethanol solutions depleted of divalent cations (Riazance-Lawrence and Johnson, 1992). We were surprised to induce the A-form of poly(dA-dC) · poly(dG-dT) without any purification of the polynucleotide. We have obtained the same A-form with a sample of poly(dA-dC) · poly(dG-dT) purified as reported before (Riazance-Lawrence and Johnson, 1992).

The A-form of poly(dA-dC) · poly(dG-dT) was, however, observed only at low temperatures (Fig. 1, *inset B*). The CD spectrum of the A-form was temperature-independent within 0–7°C, but then started to decrease gradually until the spectrum with the two positive bands appeared, which was observed in the absence of ethanol at molar sodium perchlorate concentrations (Fig. 1). The polynucleotide only denatured at 30°C, so the A-form did not denature directly but first transformed into the conformer showing the two positive bands in the long-wavelength region of the CD spectrum. The temperature-induced process, preceding the polynucleotide denaturation, is not cooperative and the CD spectra do not intersect in isoelliptic points.

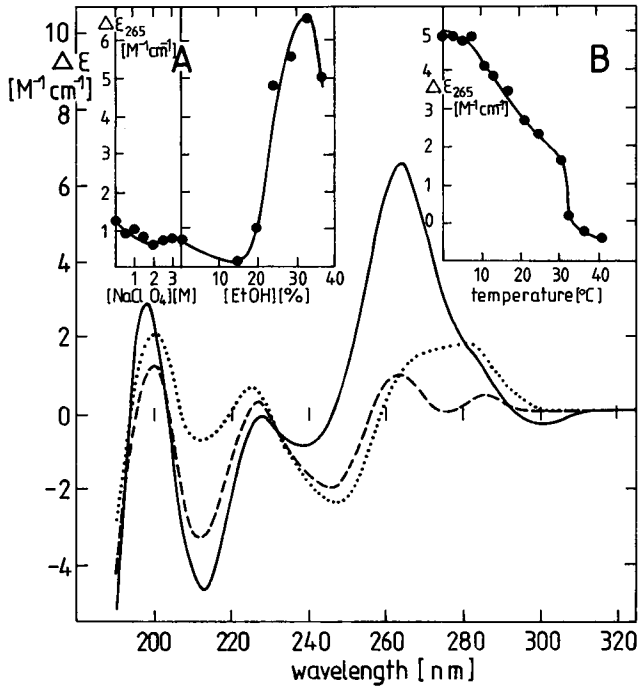

FIGURE 1 CD spectra of the B- and A-forms of poly(dA-dC) · poly(dG-dT) in $NaClO_4$ + ethanol solutions. Temperature, 0°C. · · · · ·, no $NaClO_4$, no ethanol (B-form); – – –, 3 M $NaClO_4$ + 19.6% ethanol (nonstandard B-form); ——, 3 M $NaClO_4$ + 33.1% ethanol (A-form). (*Inset A*) The course of changes in the CD spectra of poly(dA-dC) · poly(dG-dT) caused (*left*) by $NaClO_4$ (no ethanol) and (*right*) by an increasing ethanol concentration at the constant 3 M $NaClO_4$ (solid $NaClO_4$ was added into the solution to compensate for the dilution caused by the added ethanol). The latter changes reflect the B-A transition of the polynucleotide. The changes were monitored at 265 nm. (*Inset B*) Temperature-induced changes in the CD spectrum of the A-form of poly(dA-dC) · poly(dG-dT) in 2.8 M $NaClO_4$ and 32.4% EtOH monitored at 265 nm.

A-Z transition of poly(dA-dC) · poly(dG-dT)

Poly(dA-dC) · poly(dG-dT) transforms into the Z-form instead of the A-form if the polynucleotide sample contains Ni^{2+} cations (Riazance-Lawrence and Johnson, 1992). This inspired us to induce the A-Z transition of poly(dA-dC) · poly(dG-dT) by $NiCl_2$ (Fig. 2). At low temperatures required for the A-form stability (Fig. 1 *B*), $NiCl_2$ indeed induced a highly cooperative transition of the polynucleotide into the Z-form that was accompanied by an appearance of a negative band at 290 nm, a decrease of the positive band at 260 nm, and a blue shift of the short-wavelength negative band (Fig. 2). The transition midpoint was about 0.3 mM $NiCl_2$ (2.5 Ni^{2+}/P; Fig. 2, *inset*). The CD spectra intersected in isoelliptic points at 245 and 212 nm during the transition.

B-Z transition of poly(dA-dC) · poly(dG-dT)

Poly(dA-dC) · poly(dG-dT) isomerized into the Z-form even without adding divalent cations. However, this transition required very high sodium perchlorate and relatively low ethanol concentrations. For example, the transition took place either in 5–6 M sodium perchlorate in the presence of 10% ethanol (Fig. 3, *inset A*), or in 4–5 M sodium perchlorate in 15% ethanol (Fig. 4, *inset B*). The B-Z transition was two-state and slow. However, the Z-form arose only at temperatures around 15°C. Temperature lowering induced a polynucleotide isomerization (the spectra recorded between 5 and 15°C had isodichroic points) back to the B-form (Fig. 3, *inset B*), whereas higher temperatures denatured the polynucleotide duplex.

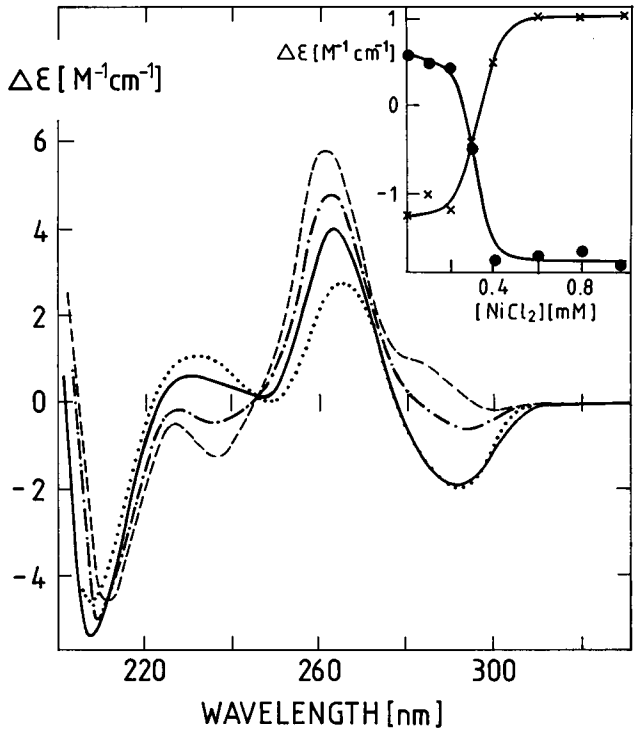

FIGURE 2 $NiCl_2$-induced A-Z transition of poly(dA-dC) · poly(dG-dT) in 3 M $NaClO_4$ and 28% EtOH at −2°C. – – –, 0.2; – · – ·, 0.3; ——, 0.4; · · · · ·, 1 mM $NiCl_2$. (*Inset*) The A-Z transition monitored by ellipticity changes at 290 (●) and 235 (×) nm.

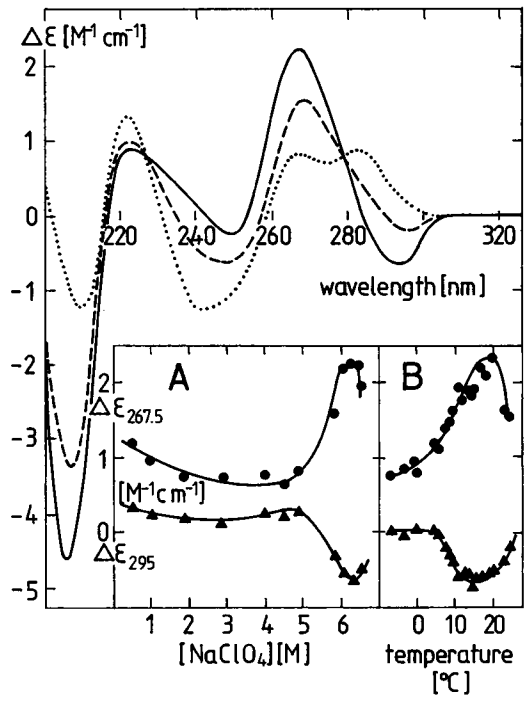

FIGURE 3 CD spectra reflecting the transition of poly(dA-dC)·poly(dG-dT) from the B-form to the Z-form in $NaClO_4$-ethanol solutions. $NaClO_4$ was added into the polynucleotide solution containing a constant 10% concentration of ethanol (the ethanol was added to the solution to compensate for the dilution caused by adding the solid salt). · · · · ·, 10% ethanol + 4.9 M $NaClO_4$; – – –, 10% ethanol + 5.8 M $NaClO_4$; ——, 10% ethanol + 6.3 M $NaClO_4$. Temperature, 20°C. (*Inset A*) CD changes of poly(dA-dC) · poly(dG-dT) caused by $NaClO_4$ in the presence of a constant 10% concentration of ethanol, monitored at 267.5 nm and 295 nm. Temperature, 20°C. (*Inset B*) Temperature-induced CD changes of poly(dA-dC) · poly(dG-dT) in 10% ethanol and 6.3 M $NaClO_4$, monitored as in *inset A*.

The shape of the CD spectrum of the perchlorate-ethanol Z-form of poly(dA-dC) · poly(dG-dT) induced without the divalent cations is the same as in Fig. 2 and as reported in the literature (Jovin et al., 1983; Riazance- Lawrence and Johnson, 1992), but the band amplitudes are smaller. The transition, however, has properties which indicate without any doubt that the Z-form is the arising conformation. It is probable that the transition is not complete at 15°C because the conditions are too close to polynucleotide denaturation.

Z′-form of poly(dA-dC) · poly(dG-dT)

We supposed the transition would be completed after the addition of $NiCl_2$. However, the addition of $NiCl_2$ induced CD changes opposite those expected (Fig. 4). A CD spectrum finally arose that was approximately a mirror image above 240 nm to that of the Z-form while the short wavelength region remained negative (Fig. 4). Cooperativity of the spectral changes was low (Fig. 4, *inset C*) and the kinetics was fast, indicating that the process proceeded within the Z-form conformational family. The arising conformer was extremely unstacked, as suggested by UV absorption spectroscopy (see below, Fig. 4, *inset A*).

Addition of $NaClO_4$ increased the UV absorption maximum of poly(dA-dC) · poly(dG-dT), even within its B-form. Formation of the Z-form was accompanied by a further increase of the absorption maximum and a slight shoulder appearance on the long-wavelength part of the UV spectrum, as observed during the B-Z transition of poly(dG-dC) · poly(dG-dC) (Pohl and Jovin, 1972). Addition of $NiCl_2$ resulted in a further hyperchromicity increase, but the UV absorption decreased when the CD spectrum started inverting. Simultaneously, the long-wavelength shoulder of the absorption band became more distinct (Fig. 4, *inset A*). These properties also support the view that $NiCl_2$ induces a transition of poly(dA-dC) · poly(dG-dT) within the Z-DNA family of conformations. The course of the transition and the solvent conditions are analogous to those inducing the Z-Z′ transition with poly(dG-dC) · poly(dG-dC) (Pohl, 1976; Hall and Maestre, 1984; Harder and Johnson, 1990), poly(dG-methyl^{5}dC) · poly(dG-methyl^{5}dC) (Zhong and Johnson, 1990; Sági et al., 1991), and poly(dG-ethyl^{5}dC) · poly(dG-ethyl^{5}dC) (Sági et al., 1991). CD spectra of these Z′-forms characteristically start with a positive band at long wavelengths and, in particular, have a deep negative band close to 200 nm, which is exhibited by left-handed Z-forms (Riazance et al., 1987). Consequently, we call this $NiCl_2$-stabilized conformation of poly(dA-dC) · poly(dG-dT) the Z′-form.

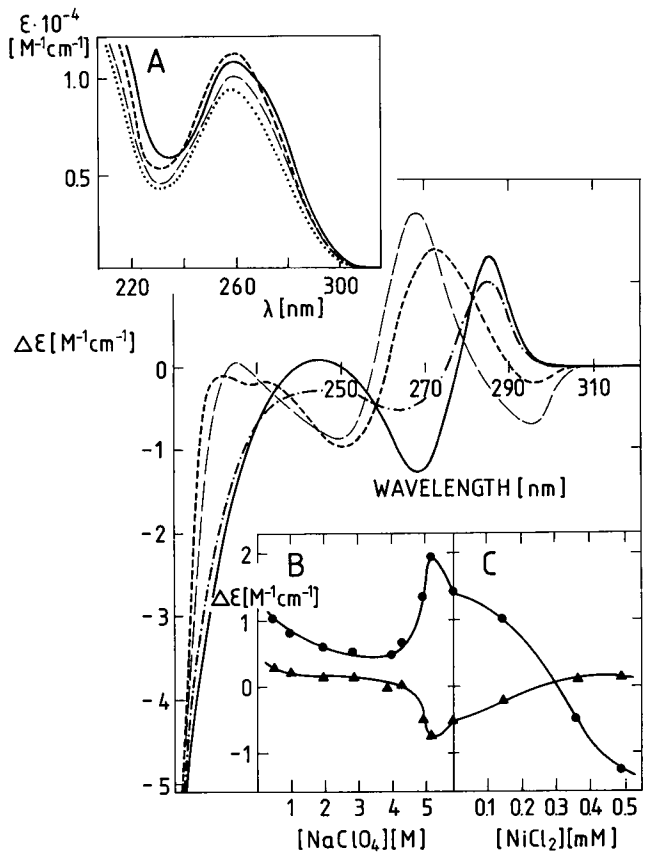

FIGURE 4 Changes in the CD spectra of poly(dA-dC) · poly(dG-dT) in $NaClO_4$ + ethanol upon addition of $NiCl_2$. Temperature, 20°C. – – –, 15% ethanol, 5.2 M $NaClO_4$, no $NiCl_2$ (Z-form); 15% ethanol, 5.9 M $NaClO_4$ plus 0.14 mM (- - - -), 0.36 mM (– · – ·), or 0.49 mM (——) $NiCl_2$. (*Inset A*) UV absorption spectra taken under the same conditions as in the main figure (CD and UV spectra measured under the same conditions are drawn by the same line types). · · · · ·, 15% ethanol and 4.3 M $NaClO_4$ (the last B-form before the transition to Z, see *inset B*). (*Inset B*) $NaClO_4$-induced CD changes of poly(dA-dC) · poly(dG-dT) at constant 15% ethanol, monitored at (circles) 267.5 nm (●) and 295 nm (▲). (*Inset C*) $NiCl_2$-induced CD changes of poly(dA-dC) · poly(dG-dT) in 15% ethanol and 5.9 M $NaClO_4$, monitored as in *inset B*.

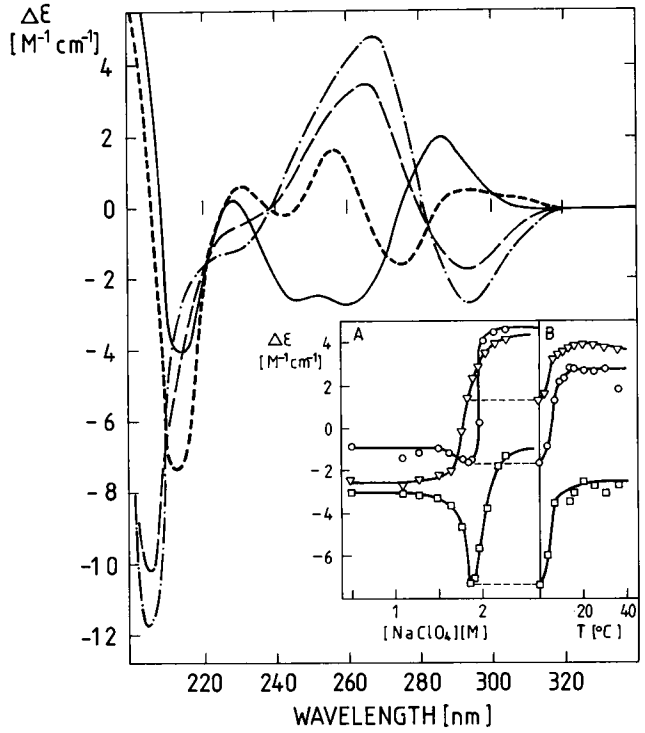

FIGURE 5 Changes in the CD spectra of poly(dA-br^{5}dC) · poly(dG-dT) in $NaClO_4$ and ethanol solutions. ——, Tris-HCl, pH 7.5, 20% ethanol, temperature 0°C (B-form); 20.5% ethanol + 1.9 M $NaClO_4$, measured at 0 (A-form) (- - - -), 7.2 (– – –), and 16.0 (– · – ·) °C (Z-form). (*Inset A*) $NaClO_4$-induced B-A and A-Z transitions of poly(dA-br^{5}dC) · poly(dG-dT) at a constant 20% concentration of ethanol at 0°C monitored at 275 (○), 260 (▽), and 215 (□) nm. (*Inset B*) Temperature-induced A-Z transition of poly(dA-br^{5}dC) · poly(dG-dT) in 20% ethanol and 1.9 M $NaClO_4$ monitored as in *inset A*.

Poly(dA-br^{5}dC) · poly(dG-dT)

Bromination at the 5-position of cytosine stabilizes the Z-form (Jovin and Soumpasis, 1987). Poly(dA-br^{5}dC) · poly(dG-dT) adopts the Z-form in perchlorate without ethanol (Jovin et al., 1983). The transition midpoint is 3.4 M $NaClO_4$. At room temperature, ethanol substantially shifts the transition midpoint toward lower perchlorate concentrations.

Another process, however, took place at low temperatures (Fig. 5). The addition of perchlorate at 0°C and a constant 20% ethanol (higher ethanol concentrations aggregated the polynucleotide) gave rise, within 1.5–1.9 M $NaClO_4$, to a cooperative appearance (Fig. 5, *inset A*) of a CD spectrum that arose at high trifluorethanol concentrations as well (not shown). It therefore seems that an A-form was adopted by poly(dA-br^{5}dC) · poly(dG-dT). This interpretation is supported by the presence of the negative CD band at 213 nm. On the other hand, the CD spectrum lacks the large positive band at 260 nm, which also is characteristic for the A-form. However, a very similar CD spectrum is also displayed (Vorlíčková et al., 1992) by the A-form of poly(dA-br^{5}dU) · poly(dA-br^{5}dU) at high trifluorethanol concentrations. This latter polynucleotide has been shown to adopt a very regular A-form in dehydrated fibers (Chandrasekaran et al., 1981).

Further addition of $NaClO_4$ at 20% ethanol and at low temperature led to a cooperative and slow switch of the polynucleotide to the Z-form (Fig. 5, *inset A*). The same process could be induced by increasing the temperature, which had the same effect as the addition of $NaClO_4$ (Fig. 5 and Fig. 5, *inset B*). The temperature-induced A-Z transition was not reversible. The Z-form seems to be a very stable and kinetically trapped conformation of poly(dA-br^{5}dC) · poly(dG-dT). Adding $NiCl_2$ did not induce the Z-Z′ transition of this polynucleotide.

The B-A transition of poly(dA-dT) · poly(dA-dT)

We have been studying conformational properties of poly (dA-dT) · poly(dA-dT) for many years (Vorlíčková et al., 1983; Vorlíčková and Kypr, 1985; Kypr and Vorlíčková,

1988; Kypr et al., 1994). That is why we were interested in the behavior of this polynucleotide in the molar sodium perchlorate-low ethanol solutions. The B-A transition of poly(dA-dT) · poly(dA-dT) was observed (Fig. 6) under conditions similar to those above with poly(dA-dC) · poly(dG-dT). The transition was cooperative (isoelliptic points at 224 nm and 274 nm) and reversible, and it showed no kinetics detectable by CD. The resulting CD spectrum was the same as that induced by high-ethanol concentrations in the absence of sodium perchlorate (Vorlíčková et al., 1982) and was quite different from the CD spectrum of the psi-form of the polynucleotide (see below). As with poly(dA-dC) · poly(dG-dT), the A-form of poly(dA-dT) · poly(dA-dT) was only stable at temperatures close to 0°C. The polynucleotide isomerized, through a two-state process (isodichroic points at 271 and 227 nm), into the B-form at 0–12°C (Fig. 7) and then denatured at still higher temperatures (T_m = 17°C). This follows from Fig. 7, because there are two consecutive processes characterized by different isodichroic points, taking place at different temperatures. The second process is accompanied by increasing hyperchromicity (Fig. 7 *B*), whereas the UV absorption spectrum does not change during the first process. Both processes, i.e., the denaturation and the temperature-induced A-B transition (Fig. 7), are fully reversible (Fig. 7 *B*).

The A-form of poly(dA-dT) · poly(dA-dT) arises within broader boundaries of the solution conditions than the A-form of poly(dA-dC) · poly(dG-dT). Fig. 8 is a map of the conformations of poly(dA-dT) · poly(dA-dT) at −2–0°C at varying concentrations of ethanol and sodium perchlorate. Triangles indicate that the polynucleotide is the A-form, and the attached numbers refer to the ellipticity values of its characteristic positive maximum at 260 nm. The triangles lacking the attached number refer to the CD spectra with an A-form shape whose positive ellipticity band amplitudes are, however, smaller than 5 $M^{-1}cm^{-1}$. The diagram is, surprisingly, quite independent of the absence or presence of EDTA in the polynucleotide solution. Fig. 8 shows that poly(dA-dT) · poly(dA-dT) adopts the A-form in two regions of the solvent composition. The first region is located around 30% ethanol and the second above 50% ethanol. The diagram also contains the A-form induced by almost 70% ethanol in the absence of sodium perchlorate, as known from previous studies (Vorlíčková et al., 1982). Very high concentrations of sodium perchlorate denature poly(dA-dT) · poly(dA-dT), whereas medium sodium perchlorate and high ethanol concentrations cause an irreversible polynucleotide aggregation (Fig. 8, points under the diagonal). The left bottom part of the diagram reflects B-form, and the polynucleotide forms psi condensates in the presence of sodium perchlorate concentrations higher than 1 M combined with ethanol concentrations higher than 25%.

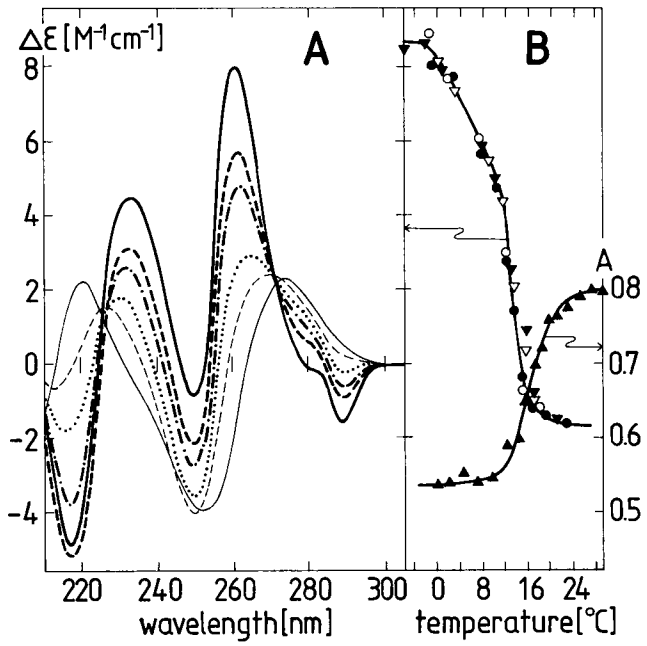

FIGURE 7 Temperature-induced changes in the CD spectra of poly(dA-dT) · poly(dA-dT) in 30.6% ethanol and 2.6 M $NaClO_4$. (*A*) Temperature: ——, −1.5; – – –, 8.0; – · – ·, 10.5; · · · · ·, 12.2°C (these thick-line spectra reflect the A-B transition of the polynucleotide); – – –, 13.7; ——, 23.4°C (these thin-line spectra reflect the polynucleotide denaturation). (*B*) Temperature-induced changes in the CD spectrum of poly(dA-dT) · (dA-dT) in 30.6% ethanol plus 2.6 M $NaClO_4$ (●, ○) and in 29.3% ethanol plus 2.5 M $NaClO_4$ (▼, ▽), both monitored at 261 nm. Closed and open symbols correspond to an increasing and decreasing temperature, respectively. ▲, A parallel UV absorption melting curve in 29.3% ethanol plus 2.5 M $NaClO_4$.

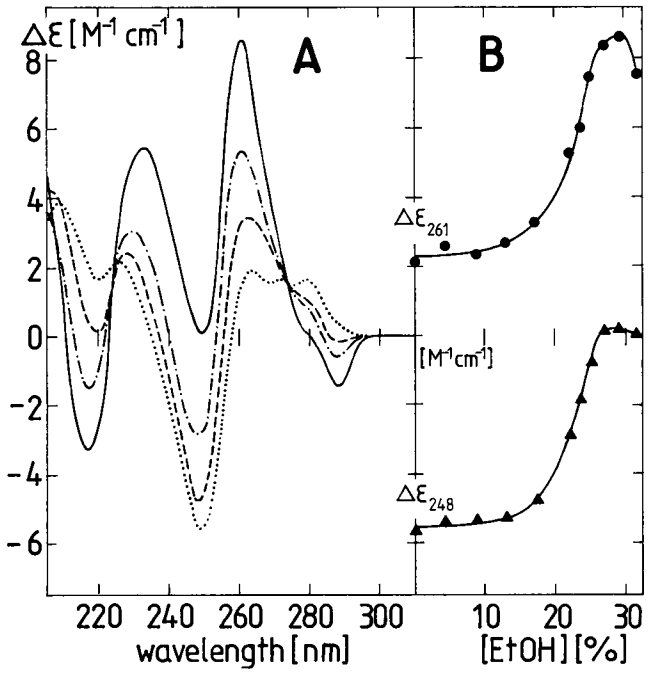

FIGURE 6 (*A*) CD spectra reflecting the B-A transition of poly(dA-dT) · poly(dA-dT) in aqueous $NaClO_4$-ethanol solutions. · · · · ·, no $NaClO_4$, no ethanol, temperature 0°C; 3 M $NaClO_4$ plus 17.4% (– – –), 22.3% (– · – ·), and 27.3% (——) ethanol. Temperature, −4°C. (*B*) The ethanol-induced B-A transition of poly(dA-dT) · poly(dA-dT) at the constant 3 M $NaClO_4$, monitored by ellipticity at 261 nm and 248 nm. Temperature, −4°C.

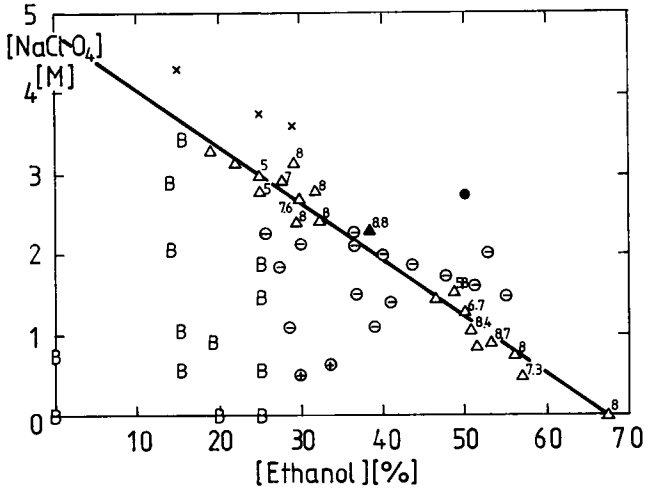

FIGURE 8 Conformational diagram of poly(dA-dT) · poly(dA-dT) in the $NaClO_4$-ethanol solutions. Temperature was within −2 and 0°C. B, conditions stabilizing B-form of poly (dA-dT) · poly(dA-dT); △, conditions stabilizing A-form (attached numbers refer to the ellipticity of its positive CD maximum). Triangles without attached numbers correspond to A-type CD spectra with positive maximum ellipticities smaller than 5. ▲, a condensed A-form giving rise to light scattering. Circles containing minus and plus signs indicate psi(−) and psi(+) condensation, respectively. ●, irreversible aggregation; ×, denaturation.

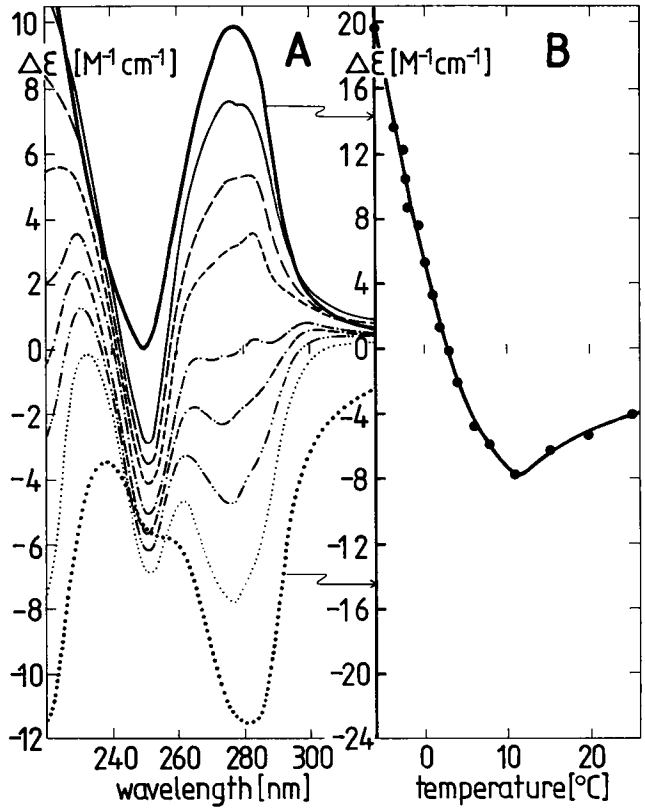

FIGURE 9 CD spectra reflecting the temperature-controlled psi-type condensation of poly(dA-dT) · poly(dA-dT) in ethanol-$NaClO_4$ solutions. (*A*) 33.6% ethanol plus 0.61 M $NaClO_4$. Temperatures: (the uninterrupted bold line) −6.0°C. Thin lines: ——, −0.6; — —, 0.2; – – –, 1.5; – · – ·, 3.3; · – – ·, 4.2; – · · –, 6.3; · · · · ·, 11.0°C. Bold dotted line, 30.1% ethanol plus 2.1 M $NaClO_4$, temperature −1.5°C. The spectra drawn in bold belong to the ellipticity scale on the right side of the figure. (*B*) Temperature-induced changes in the poly(dA-dT) · poly(dA-dT) condensation as monitored by ellipticity at 277 nm.

Psi condensates of poly(dA-dT) · poly(dA-dT)

The condensation separates the region of A-form stability into the two parts described above. Psi condensates of poly(dA-dT) · poly(dA-dT) can be transformed into the A-form by appropriate changes in the solution conditions, but the transformation takes a long time and the decondensation usually is not complete, i.e., the resulting A-type CD spectrum shows a small residual light scattering. However, the A-form of poly(dA-dT) · poly(dA-dT) showed no light scattering if the region of psi formation was avoided in the course of the sample preparation, for which the diagram (Fig. 8) was useful.

Psi condensation of poly(dA-dT) · poly(dA-dT) has previously been studied in detail when hexaminecobalt was the inducing agent (Thomas and Bloomfield, 1985; Shin et al., 1986). We show here (Fig. 9) that poly(dA-dT) · poly(dA-dT) forms both psi(−) and psi(+) condensates in the present perchlorate-ethanol solvent as well. The psi(−) condensates, which are more frequent with this polynucleotide, display relatively weak ellipticity values (the maximum ellipticity was about $-20\ M^{-1}cm^{-1}$, but solvent conditions very distinct from the diagram diagonal were not examined). Another characteristic feature of the psi condensates is a long kinetics of formation and light scattering, giving rise to a nonzero ellipticity in the wavelength region above 300 nm, where the canonical bases of DNA no longer absorb light. The weak psi(−) CD spectra are quite different from the CD spectrum of the X-form of poly(dA-dT) · poly(dA-dT) (Vorlíčková et al., 1983; Vorlíčková and Kypr, 1985). Low sodium perchlorate concentrations and about 30% ethanol give psi(+) condensates, whereas a temperature increase above zero transforms psi(+) into weak psi(−) (Fig. 9). This transformation is completely reversible.

Transition of poly(dA-dT) · poly(dA-dT) into Z'-form

We tried to transform poly(dA-dT) · poly(dA-dT) into Z-form in the sodium perchlorate-ethanol solutions, but the attempts to get the CD spectrum of the Z-form induced by NaCl + $NiCl_2$ (Bourtayre et al., 1987) failed. Guided by the behavior of poly (dA-dC) · poly(dG-dT), we mainly searched for the Z-form of poly(dA-dT) · poly(dA-dT) at low ethanol concentrations, where the A-form could not compete. Additions of $NaClO_4$ (at a constant 15% ethanol and 0°C) changed (Fig. 10) the CD spectrum of poly(dA-dT) · poly(dA-dT) toward the A-form spectrum, as in Fig. 6. However, the thermolabile duplex of poly(dA-dT) · poly(dA-dT) denatured upon increasing the perchlorate concentration above 4.3 M. The NaCl + $NiCl_2$-

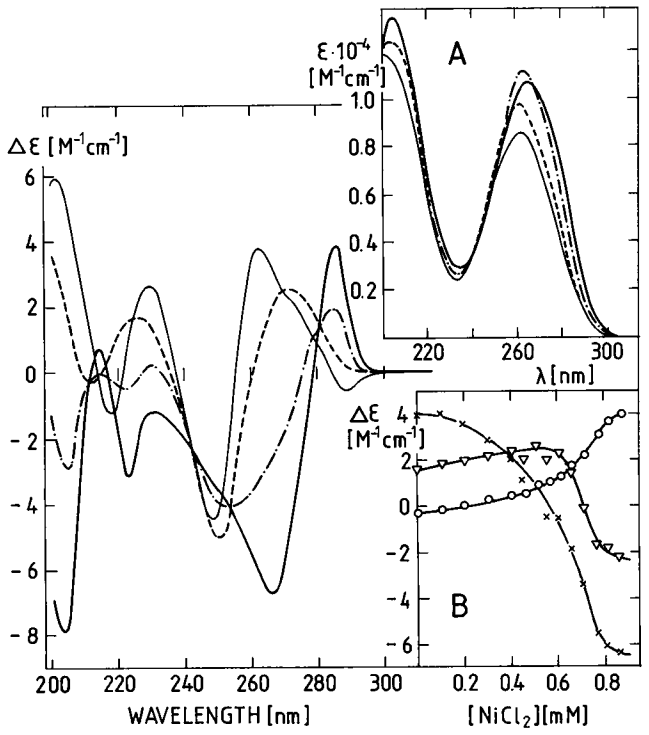

FIGURE 10 Changes in the CD spectra of poly(dA-dT) · poly(dA-dT) in $NaClO_4$ + ethanol upon addition of $NiCl_2$. 15% ethanol, 4.3 M $NaClO_4$ plus 0 (thin uninterrupted line), 0.46 (– – –), 0.71 (– · – · –), and 0.87 (——) mM $NiCl_2$. Temperature, −2°C. (*Inset A*) UV spectra measured under the same conditions as the CD spectra drawn by the same line type. (*Inset B*) $NiCl_2$-induced CD changes of poly(dA-dT) · poly(dA-dT) in 15% ethanol + 4.3 M $NaClO_4$, monitored at 262.5 (×), 275 (▽), and 285 (○) nm.

induced Z-form did not appear even in the presence of $NiCl_2$ in perchlorate-ethanol solutions.

In analogy with poly(dA-dC) · poly(dG-dT), $NiCl_2$ hindered the isomerization of poly(dA-dT) · poly(dA-dT) to the A-form (starting from 0.06 mM concentration); another process took place instead (Fig. 10). The first additions of $NiCl_2$ shifted the positive CD maximum of the polynucleotide toward longer wavelengths, suggesting its denaturation. The denaturation was also indicated by the large increase of the UV absorption maximum (Fig. 10, *inset A*), which still increased upon further additions of $NiCl_2$. However, starting with 0.6 mM $NiCl_2$ (Fig. 10 *B*), a conformer was stabilized that had a CD spectrum that was more or less an inversion of the CD spectrum observed in the absence of $NiCl_2$ (Fig. 10). The spectrum was similar to that of the Z′-form of poly(dA-dC) · poly(dG-dT) (Fig. 4). It also had the characteristic deep negative band at about 204 nm, like the Z-forms of poly(dA-dC) · poly(dG-dT), poly(dA-br^{5}dC) · poly(dG-br^{5}dU) (Riazance-Lawrence et al., 1987), and poly(dA-br^{5}dC) · poly(dG-dT) (Fig. 5). The $NiCl_2$-induced transition had a long kinetics and seemed to be of a two-state nature. During the time-dependent process, the UV absorption band slightly diminished (Fig. 10, *inset A*) and shifted toward longer wavelengths, as is usual with Z-forms. Further additions of $NiCl_2$ exceeding a 0.9 mM concentration condensed the sample.

The Z′-form of poly(dA-dT) · poly(dA-dT) is very unstacked, but it differs from the unstacked P-form observed before (Zehfus and Johnson, 1981, 1984). We examined the behavior of poly(dA-dT) · poly(dA-dT) in a wide range of methanol-SSC/20-ethanol solutions inducing the P-form in calf thymus DNA and never observed the Z′-form CD spectrum. However, we induced the P-form of poly (dA-dT) · poly(dA-dT) in methanol-SSC/20-ethanol (65–5-30). The CD spectrum of the P-form was similar to that of the denatured polynucleotide and to the CD spectrum obtained after the first additions of $NiCl_2$ (Fig. 10). Thus the conformation from which poly(dA-dT) · poly(dA-dT) slowly isomerized into the Z′-form is either a denatured state or the P-form.

Poly(dI-dC) · poly(dI-dC)

Poly(dI-dC) · poly(dI-dC) provides a CD spectrum and an x-ray fiber diffraction pattern, both of which are anomalous, indicating a peculiar polynucleotide conformation (Mitsui et al., 1970). The CD spectrum starts with the unusual Z-like negative band on the long wavelength side to end with a deep negative band at 204 nm (Fig. 11 *A*). Both ethanol and $NaClO_4$ (Fig. 11) decrease all negative CD bands of

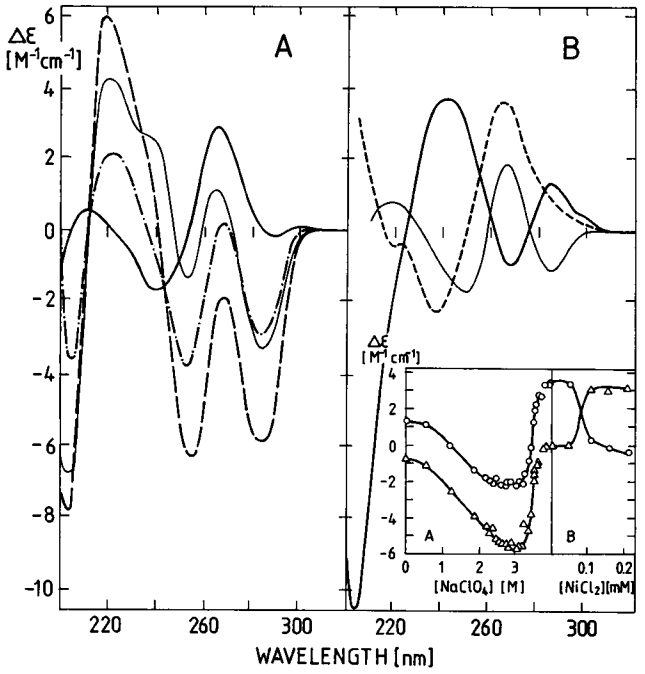

FIGURE 11 Changes in the CD spectra of poly(dI-dC) · poly(dI-dC) in $NaClO_4$ + ethanol, and upon addition of $NiCl_2$. The polynucleotide was dissolved in 10 mM Tris-HCl, pH 7.5, plus 0.1 mM CDTA, and measured at −2°C. (*A*) constant 15% ethanol plus 0 (thin uninterrupted line), 3.10 (– – –), 3.43 (– · – · –), and 3.70 (——) M $NaClO_4$. (*B*) constant 15% ethanol and 3.6 M $NaClO_4$ (thin uninterrupted line), 4 M $NaClO_4$ (– – –), 4 M $NaClO_4$ plus 0.2 mM $NiCl_2$ (——). (*Inset A*) $NaClO_4$-induced changes in the CD spectra of poly (dI-dC) · poly(dI-dC) in 15% ethanol at −2°C, monitored at 265 nm (○) and 250 nm (△). (*Inset B*) $NiCl_2$-induced changes in the CD spectra of poly(dI-dC) · poly(dI-dC) in 15% ethanol + 4 M $NaClO_4$ at −2°C, monitored as in *inset A*.

poly(dI-dC) · poly(dI-dC). At constant 15% ethanol and a $NaClO_4$ concentration higher than 3 M, a sudden cooperative two-state transition takes place (Fig. 11, *A* and *inset A*), to give a CD spectrum observed at high ethanol concentrations (Vorlíčková and Sági, 1991) or upon peptide binding (Rao et al., 1987) when poly(dI-dC) · poly(dI-dC) adopts the canonical B-form.

In the presence of ethanol plus 4 M $NaClO_4$, the CD spectrum suggests denaturation of poly(dI-dC) · poly(dI-dC) (Vorlíčková and Sági, 1991; Fig. 11 *B*). Addition of decimillimolar $NiCl_2$, however, transforms poly(dI-dC) · poly (dI-dC) into a Z-form (Fig. 11, *B* and *inset B*), as originally observed by CD spectroscopy in NaCl + $NiCl_2$ (Vorlíčková and Sági, 1991) and then confirmed by Raman spectroscopy (Miskovsky et al., 1993). The Z-form CD spectrum contains a positive band at 285 nm, a negative one at 270 nm, a strong positive band at 240 nm, and a deep negative band at 203 nm. The $NiCl_2$-induced inversion of the CD spectrum, accompanying formation of the Z-form (Fig. 11, *B* and *inset B*), was slow. Similar inducing conditions and positions of the CD bands with the Z′-forms of poly(dA-dT) · poly(dA-dT) and poly(dA-dC) · poly(dG-dT) suggest that the Z-form of poly(dI-dC) · poly(dI-dC) is in fact the Z′-form.

Poly(dI-dC) · poly(dI-dC) isomerizes into the A-form in aqueous trifluoroethanol solutions (Vorlíčková and Sági, 1991), but we did not manage to induce the A-form in $NaClO_4$-ethanol, although the sample was thoroughly depleted of divalent cations by the procedure reported in the literature (Riazance-Lawrence and Johnson, 1992). The polynucleotide condensed into psi(+) at relatively low (0.8 M) $NaClO_4$ concentrations in 30% ethanol and low temperatures.

Poly(dI-br^{5}dC) · poly(dI-br^{5}dC)

The Z-form is adopted by this polynucleotide at high NaCl (Patel et al., 1979; Hartman et al., 1982) or alcohol (Vorlíčková and Sági, 1991) concentrations. $NaClO_4$ also induces the Z-form in poly(dI-br^{5}dC) · poly(dI-br^{5}dC) (Fig. 12). The transition takes place at 2.1 M $NaClO_4$ (Fig. 12, *inset*); it is very cooperative and slow. The transition is shifted to 1.2 M $NaClO_4$ in 15% ethanol (Fig. 12, *inset*), and the resulting CD spectrum lacks the long-wavelength negative band observed in the $NaClO_4$ alone (Fig. 12). Both of the Z-form CD spectra were also observed at high ethanol or trifluoroethanol concentrations (Vorlíčková and Sági, 1991). Changes in ethanol concentration induced noncooperative transitions between the two Z-forms (Vorlíčková and Sági, 1991). The CD spectrum, not containing the negative long-wavelength band, has the ellipticity maxima and minima at the same wavelengths as the Z′-forms of poly(dA-dC) · poly(dG-dT) and poly(dA-dT) · poly(dA-dT), although they lack the strong positive band at 240 nm specific for the Z-forms of the poly(dI-dC) · poly(dI-dC) family of DNAs.

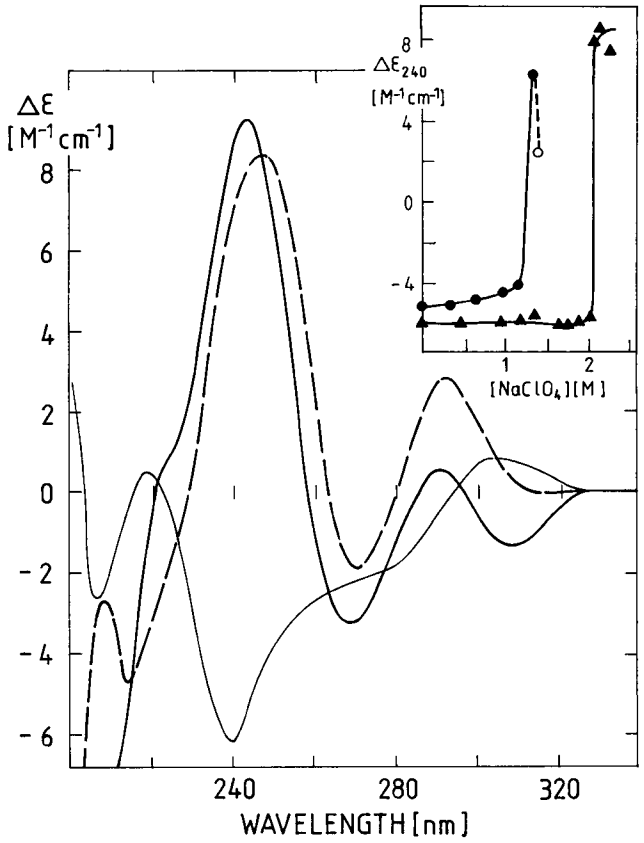

FIGURE 12 CD spectra of poly(dI-br^{5}dC) · poly(dI-br^{5}dC) in the presence of $NaClO_4$ + ethanol, temperature 21°C, 10 mM Tris-HCl, 0.02 mM EDTA, pH 7.48. Thin uninterrupted line, no $NaClO_4$, no ethanol; ——, 2.2 M $NaClO_4$, – – –, 2.2 M $NaClO_4$ plus 5% ethanol. (*Inset*) $NaClO_4$-induced transition of poly(dI-br^{5}dC) · poly(dI-br^{5}dC) in the absence (▼) and presence (●) of 15% ethanol. ○, aggregation.

DISCUSSION

This work describes conformational properties of five alternating purine-pyrimidine DNAs in the presence of molar sodium perchlorate and moderate (up to about 30%) ethanol concentrations to show that this solvent can stabilize not only the Z-form but also the A-form. This solvent thus represents an extension of the spectrum of solvents inducing the non-B duplexes. The fact that different solvents induce the same non-B conformations suggests that the Z-form and the A-form are not artefacts of crystallization, DNA condensation in a particular solvent, or CD spectroscopy. Moreover, the existence of several solvents or factors stabilizing the same conformation will help to identify the forces standing behind the stability of the non-B conformations. It is evident, for example, that molar concentrations of the perchlorate anion exert the same effect on DNA conformation as the increase of ethanol concentration from 10–30% to 75%. This effect is interesting because the perchlorate anions hardly bind to the polyanionic DNA. Rather, they influence DNA indirectly by affecting water structure and DNA hydration (Breslow and Guo, 1990). The high ethanol concentrations probably change DNA hydration in the same

way, which indicates that the hydration is a key determinant of DNA conformation.

We demonstrate here that poly(dA-dC) · poly(dG-dT) can be transformed to both the A-form and the Z-form in the perchlorate-ethanol solvent, whereas the resulting conformer depends on solution conditions. In the absence of divalent cations, the Z-form requires very high sodium perchlorate and low ethanol concentrations whereas the A-form is stable at moderate sodium perchlorate and moderate ethanol concentrations. Moreover, the Z-form is not formed at low temperatures which, on the contrary, are needed for A-form stability.

In the presence of $NiCl_2$, the B-A transition of poly(dA-dC) · poly(dG-dT) is replaced by the B-Z transition. Addition of $NiCl_2$ to the A-form of poly(dA-dC) · poly(dG-dT) leads to the A-Z transition, whereas the addition of $NiCl_2$ to the Z-form of poly(dA-dC) · poly(dG-dT) inverts its CD spectrum, presumably reflecting the Z-Z′ transition. This conclusion is supported by the UV absorption properties, the low cooperativity and fast kinetics of the transition, the deep negative CD signal in the vicinity of 200 nm, and the analogy of the solvent conditions inducing the Z-Z′ transition with poly(dG-dC) · poly(dG-dC) (Pohl, 1976; Hall and Maestre, 1984; Harder and Johnson, 1990), poly(dG-methyl^{5}dC) · poly(dG-methyl^{5}dC) (Zhong and Johnson, 1990; Sági et al., 1991), and poly(dG-ethyl^{5}dC) · poly(dG-ethyl^{5}dC) (Sági et al., 1991) at very high alcohol concentrations, or after the addition of divalent cations to their Z-forms. It was suggested (Harder and Johnson, 1990) that the arising conformation corresponded to the Z_{II}-conformation observed in crystals. X-ray diffraction studies of alternating purine-pyrimidine oligodeoxynucleotide crystals grown from various solvents revealed three variants of Z-form (Drew et al., 1980; Wang et al., 1981; Drew and Dickerson, 1981b). Studies in solution, including the present one, indicate that Z-form variability is not specific for the crystals. Therefore, the Z-forms generate a family of related but different DNA conformations like the B-forms or A-forms.

Poly(dA-dT) · poly(dA-dT) also isomerizes into the A-form under conditions stabilizing the A-form in poly(dA-dC) · poly(dG-dT). The A-form has long been thought, on the basis of its conformational properties in fibers, to be only a metastable solution conformation of poly(dA-dT) · poly(dA-dT) (Davies and Baldwin, 1963; Leslie et al., 1980). However, we showed (Vorlíčková et al., 1982, 1991b) that high ethanol concentrations induced the B-A isomerization of poly(dA-dT) · poly(dA-dT) in solution. The A-form was stable and not metastable, although within only a narrow range of solution conditions. Thus it was rather surprising to see here that the A-form of poly(dA-dT) · poly(dA-dT) could easily be induced in a wide range of perchlorate and ethanol concentrations (Fig. 8).

It is of interest that the A-form is induced by high salt concentrations because the B-A transition is hindered even by traces of divalent cations (Ivanov et al., 1974), whereas monovalent cations also destabilize the A-forms of calf thymus and other natural DNAs, even if present in submillimolar concentrations in solution (Ivanov et al., 1974). On the other hand, there is a theoretical formalism suggesting that high salt concentrations stabilize A-DNA (Soumpasis et al., 1987). So far, however, salt-induced B-A transitions have been reported with only two polynucleotides, namely poly(dG) · poly(dC) (Nishimura et al., 1986) and poly(amino^{2}dA-dT) · poly(amino^{2}dA-dT) (Borah et al., 1985). However, neither of these high-salt A-forms is without questions, because concentrated poly(dG) · poly(dC) is A-form even in the absence of high-salt or alcohol (Benevides et al., 1986; Sarma et al., 1986), whereas the putative A-form of poly(amino^{2}dA-dT) · poly(amino^{2}dA-dT) differs from the canonical A-DNA in many significant properties (Kypr et al., 1994). On the oligonucleotide level, A-DNA of the duplex of d(CCCCGGGG) is stabilized by trivalent hexamine cobalt cations in aqueous solution (Xu et al., 1993a; Xu et al., 1993b). In all of the above cases where A-DNA was induced in the absence of alcohol, amino groups completely occupied the DNA minor groove and presumably disturbed its hydration, stabilizing the B-form (Drew and Dickerson, 1981a).

DNA of the cyanophage S-2L, whose adenines are replaced by amino^{2}adenine (Kirnos et al., 1977) so that its minor groove is also completely occupied by the amino groups, easily transforms into the A-form in perchlorate-ethanol solution (M. Vorlíčková, unpublished results). In contrast to the putative A-form of poly(amino^{2}dA-dT) · poly(amino^{2}dA-dT), the CD spectrum of the A-form of S-2L DNA contains the strong positive band at 260 nm characteristic for A-DNA (Vorlíčková et al., 1991a). On the other hand, poly(dG-dC) · poly(dG-dC), i.e., another DNA with amino groups in the minor groove, did not isomerize into A-DNA in perchlorate-ethanol and only isomerized into Z-DNA and psi(−) condensates (M. Vorlíčková, unpublished results). The self-complementary 54-mer DNA fragment d(opopopopo), where o = CGCGCG and p = TATATA, behaved in a similar way. Instead of A-form, Z-form was assumed even at low temperatures and a relative excess of ethanol (M. Vorlíčková et al., unpublished results), i.e., under conditions where the A-form is preferred by some other alternating purine-pyrimidine DNAs. Thus like poly(dG-dC) · poly(dG-dC), the 54-mer duplex containing the alternating blocks of $(dC\text{-}dG)_3$ and $(dT\text{-}dA)_3$ prefers the Z-form and not the A-form.

In contrast, poly(dA-dC) · poly(dG-dT) and poly(dA-dT) · poly(dA-dT) prefer the A-form in perchlorate-ethanol solutions, whereas poly(dA-dT) · poly(dA-dT) does not adopt the Z-form observed in NaCl + $NiCl_2$, even if $NiCl_2$ is added to the perchlorate-ethanol solution. Instead, $NiCl_2$ transforms the polynucleotides into the Z′-form. Whereas poly(dA-dC) · poly(dG-dT) starts the transition to Z′-form from Z-form and the Z-Z′ transition is fast, the transitions into the Z′-form are slow with poly(dA-dT) · poly(dA-dT) and poly(dI-dC) · poly(dI-dC). The transitions to the Z′-form are preceded by substantial unstacking of bases, presumably caused by DNA dehydration induced by the per-

chlorate anions. In this way the perchlorate anions probably unwind the B-DNA double helix and promote single-stranded DNA, A-DNA or Z-DNA, depending on the solution conditions and compatibility of the DNA base sequence with the non-B conformations.

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REFERENCES

Behe, M., and G. Felsenfeld. 1981. Effects of methylation on a synthetic polynucleotide: the B-Z transition in poly(dG-m^5 dC) · poly(dG-m^5dC). *Proc. Natl. Acad. Sci. USA.* 78:1619–1623.

Benevides, J. M., A. H.-J. Wang, A. Rich, Y. Kyogoku, G. A. van der Marel, J. H. van Boom, and G. J. Thomas, Jr. 1986. Raman spectra of single crystals of r(GCG)d(CGC) and d(CCCCGGGG) as models for A-DNA, their structure transitions in aqueous solution, and comparison with double-helical poly(dG) · poly(dC). *Biochem. USA.* 25:41–50.

Bernués, J., R. Beltrán, J. M. Casasnovas, and F. Azorín. 1990. DNA-sequence and metal-ion specificity of the formation of H-DNA. *Nucleic Acids Res.* 18:4067–4073.

Bloomfield, V. A. 1991. Condensation of DNA by multivalent cations: considerations on mechanism. *Biopolymers.* 31:1471–1481.

Borah, B., J. S. Cohen, F. B. Howard, and H. T. Miles. 1985. Poly(amino^{2}dA-dT): two-dimensional NMR shows a B-to-A conversion in high salt. *Biochem. USA.* 3:7456–7462.

Bourtayre, P., J. Liguier, L. Pizzorni, and E. Taillandier. 1987. Z-form of poly(dA-dT) in solution studied by CD and UV spectroscopy. *J. Biomol. Struct. Dyn.* 5:97–104.

Breslow, R., and T. Guo. 1990. Surface tension measurements show that chaotropic salting-in denaturants are not just water structure breakers. *Proc. Natl. Acad. Sci. USA.* 87:167–169.

Chandrasekaran, R., L. C. Puigjaner, S. Arnott, R. G. He, S. Brahms, and J. Brahms. 1981. The synthetic DNA duplex of poly(dA-br^{5}dU) · poly(dA-br^{5}dU) adopts an A-DNA-like structure. *J. Biomol. Struct. Dyn.* 6:715–727.

Davies, D. R., and R. L. Baldwin. 1963. X-ray studies of two synthetic DNA copolymers. *J. Mol. Biol.* 6:251–255.

Deka, R., M. D. Shriver, L. M. Yu, L. Jin, C. E. Aston, R. Chakraborty, and R. E. Ferrell. 1994. Conservation of human chromosome 13 polymorphic microsatellite $(CA)_n$ repeats in chimpanzees. *Genomics.* 22: 226–230.

Drew, H. R., and R. E. Dickerson. 1981a. Structure of a B-DNA dodecamer. III. Geometry of hydration. *J. Mol. Biol.* 151:535–556.

Drew, H. R., and R. E. Dickerson. 1981b. Conformation and dynamics in a Z-DNA tetramer. *J. Mol. Biol.* 152:723–736.

Drew, H. R., T. Takano, S. Tanaka, K. Itakura, and R. E. Dickerson. 1980. High-salt d(CGCG), a left-handed Z′-DNA double helix. *Nature.* 286: 567–573.

Gaillard, C., and F. Strauss. 1994. Association of poly(CA) · poly(TG) DNA fragments into four-stranded complexes bound by HMG 1 and 2. *Science.* 264:433–436.

Gray, D. M., and R. L. Ratliff. 1975. Circular dichroism spectra of poly(dA-dC) · poly(dG-dT), poly(rA-rC) · poly(rG-rU), and hybrids poly(dA-dC) · poly(rG-rU) and poly(rA-rC) · poly(dG-dT) in the presence of ethanol. *Biopolymers.* 14:487–498.

Hall, K. B., and M. F. Maestre. 1984. Temperature-dependent reversible transition of poly(dG-dC) · poly(dG-dC) in ethanolic and methanolic solutions. *Biopolymers.* 23:2127–2139.

Harder, M. E., and W. C. Johnson, Jr. 1990. Stabilization of the Z′-form of poly(dG-dC) · poly(dG-dC) in solution by multivalent ions relates to the Z_{II}-form in crystals. *Nucleic Acids Res.* 18:2141–2148.

Hartman, B., J. Pilet, M. Ptak, J. Ramstein, B. Malfoy, and M. Leng. 1982. The B-Z transition of poly(dI-br^{5}dC) · poly(dI-br^{5}dC). A quantitative description of the Z-form dynamic structure. *Nucleic Acids Res.* 10: 3261–3277.

Ivanov, V. I., L. Minchenkova, E. E. Minyat, M. D. Frank-Kamenetskii, and A. K. Schyolkina. 1974. The B-to-A transition of DNA in solution. *J. Mol. Biol.* 87:817–833.

Jovin, T. M., L. P. McIntosh, D. J. Arndt-Jovin, D. A. Zarling, M. Robert-Nicoud, J. H. van de Sande, K. F. Jorgenson, and F. Eckstein. 1983. Left-handed DNA: from synthetic polymers to chromosomes. *J. Biomol. Struct. Dyn.* 1:21–57.

Jovin, T. M., and D. M. Soumpasis. 1987. The transition between B-DNA and Z-DNA. *Annu. Rev. Phys. Chem.* 38:521–560.

Kirnos, M. D., I. Ya. Khudyakov, N. I. Alexandrushkina, and B. F. Vanyushin. 1977. 2-Aminoadenine is an adenine substituting base in S-2L cyanophage DNA. *Nature.* 270:369–370.

Kypr, J., H. Peňázová, J. Sági, Š. Pospíšilová, and M. Vorlíčková. 1994. UV light-induced crosslinking of the strands of poly(dA-dT) and related alternating purine-pyrimidine DNAs. *J. Biomol. Struct. Dyn.* 11: 1225–1236.

Kypr, J., J. Sági, E. Szakonyi, K. Ebinger, H. Peňázová, J. Chládková, and M. Vorlíčková. 1994. Thymine methyl groups stabilize the putative A-form of the synthetic DNA poly(amino^{2}dA-dT). *Biochem. USA.* 33: 3801–3806.

Kypr, J., and M. Vorlíčková. 1988. Conformations of DNA duplexes containing (dA-dT) sequences of bases and their possible biological significance. *In* Structure and Expression, Vol. 2, DNA and Its Drug Complexes. R. H. Sarma and M. H. Sarma, editors. Guilderland, Adenine Press. 105–121.

Leslie, A. G. W., S. Arnott, R. Chandrasekaran, and R. L. Ratliff. 1980. Polymorphism of DNA double helices. *J. Mol. Biol.* 143:49–72.

Mirau, P. A., D. R. Kearns, L. P. McIntosh, and T. M. Jovin. 1986. ^{1}H-NMR study of the dynamic properties of the B- and Z-forms of poly(dA-br^{5}dC) · poly(dG-dT). *J. Mol. Biol.* 192:633–643.

Miškovský, P., A. Tomková, L. Chinsky, and P.-Y. Turpin. 1993. Conformational transitions of poly(dI-dC) in aqueous solution as studied by ultraviolet resonance Raman spectroscopy. *J. Biomol. Struct. Dyn.* 11: 655–669.

Mitsui, Y., R. Langridge, B. E. Shortle, Ch. R. Cantor, R. C. Grant, K. Masahiko, and R. D. Wells. 1970. Physical and enzymatic studies on poly(dI-dC) · poly(dI-dC), an unusual double-helical DNA. *Nature.* 228: 1166–1169.

Nishimura, Y., C. Torigoe, and M. Tsuboi. 1986. Salt-induced B-A transition of poly(dG) · poly(dC) and the stabilization of A-form by its methylation. *Nucleic Acids Res.* 14:2737–2749.

Patel, D. J., L. L. Canuel, and F. M. Pohl. 1979. "Alternating B-DNA" conformation for the oligo(dG-dC) duplex in high-salt solution. *Proc. Natl. Acad. Sci. USA.* 76:2508–2511.

Patel, U., S. Grundfest-Broniatowski, M. Gupta, and S. Banerjee. 1994. Microsatellite instabilities at five chromosomes in primary breast tumors. *Oncogene.* 9:3695–3700.

Pohl, F. M. 1976. Polymorphism of a synthetic DNA in solution. *Nature.* 260:365–366.

Pohl, F. M., and T. M. Jovin. 1972. Salt-induced cooperative conformational change of a synthetic DNA: equilibrium and kinetic studies with poly(dG-dC) · poly(dG-dC). *J. Mol. Biol.* 67:375–396.

Rao, M. V. R., M. Atreyi, G. S. Kumar, and S. Kumar. 1987. Reversal of the long-wavelength CD band of poly(dI-dC) · poly(dI-dC) on specific interaction with the 53–58 peptide fragment of the lac repressor. *Biopolymers.* 26:329–332.

Rhyu, M.-G., W.-S. Park, and S. J. Meltzer. 1994. Microsatellite instability occurs frequently in human gastric carcinoma. *Oncogene.* 9:29–32.

Riazance, J. H., W. C. Johnson, Jr., L. P. McIntosh, and T. M. Jovin. 1987. Vacuum UV circular dichroism is diagnostic for the left-handed Z-form of poly[d(A-C) · d(G-T)] and other polydeoxynucleotides. *Nucleic Acids Res.* 15:7627–7636.

Riazance-Lawrence, J. H., and W. C. Johnson, Jr. 1992. Multivalent ions are necessary for poly(dA-dC) · poly(dG-dT) to assume the Z-form: a CD study. *Biopolymers.* 32:271–276.

Sági, J., and L. Ötvös. 1980. Modified polynucleotides . V. Slow-down of nuclease action by 5-alkyluracil containing DNAs. *Biochem. Biophys. Res. Commun.* 95:156–162.

Sági, J., M. Vorlíčková, J. Kypr, A. Szemzö, and L. Ötvös. 1991. Destabilization of the duplex and the high-salt Z-form of poly(dG-m^{5}dC) by substitution of ethyl for the 5-methyl group. *Int. J. Biol. Macromol.* 13:329–336.

Sarkar, G., C. Paynton, and S. S. Sommer. 1991. Segments containing alternating purine and pyrimidine dinucleotides: patterns of polymorphism in humans and prevalence throughout phylogeny. *Nucleic Acids Res.* 19:631–636.

Sarma, M. H., G. Gupta, and R. H. Sarma. 1986. 500-MHz ^{1}H NMR study of poly(dG) · poly(dC) in solution using one-dimensional nuclear Overhauser effect. *Biochem. USA.* 25:3659–3665.

Shin, Y. A., S. L. Feroli, and G. L. Eichhorn. 1986. Psi compaction of poly(dA-dT) · poly(dA-dT). *Biopolymers.* 25:2133–2148.

Soumpasis, D. M., J. Wiechen, and T. M. Jovin. 1987. Relative stabilities and transitions of DNA conformations in 1:1 electrolytes: a theoretical study. *J. Biomol. Struct. Dyn.* 4:535–552.

Thomas, T. J., and V. A. Bloomfield. 1985. Quasielastic laser light scattering and electron microscopy studies of the conformational transitions and condensation of poly(dA-dT) · poly(dA-dT). *Biopolymers.* 24: 2185–2194.

Uchida, T., C. Wada, C. Wang, H. Ishida, S. Egawa, E. Yokoyama, H. Ohtani, and K. Koshiba. 1995. Microsatellite instability in prostate cancer. *Oncogene.* 10:1019–1022.

Vogt, P. 1990. Potential genetic functions of tandem repeated DNA sequence blocks in the human genome are based on a higly conserved "chromatin folding code." *Hum. Genet.* 84:301–336.

Vorlíčková, M., J. Chládková, and J. Kypr. 1992. Conformational transitions of poly(dA-br^{5}dU) and poly(dA-io^{5}dU) in solution. *Nucleic Acids Res.* 20:1109–1112.

Vorlíčková, M., I. Ya. Khudyakov, I. Hejtmánková, and J. Kypr. 1991a. Circular dichroism studies of salt- and alcohol-induced conformational changes in cyanophage S-2L DNA which contains amino^{2}adenine instead of adenine. *J. Biomol. Struct. Dyn.* 9:81–85.

Vorlíčková, M., and J. Kypr. 1985. Conformational variability of poly(dA-dT) · poly(dA-dT) and some other deoxyribonucleic acids includes a novel type of double helix. *J. Biomol. Struct. Dyn.* 3:67–83.

Vorlíčková, M., and J. Sági. 1991. Transitions of poly(dI-dC), poly(dI-m^{5}dC) and poly(dI-br^{5}dC) among and within the B-, Z-, A- and X-DNA families of conformations. *Nucleic Acids Res.* 19:2343–2347.

Vorlíčková, M., J. Sági, I. Hejtmánková, and J. Kypr. 1991b. Alkyl substituent in place of the thymine methyl group controls the A-X conformational bimorphism in poly(dA-dT). *J. Biomol. Struct. Dyn.* 9:571–578.

Vorlíčková, M., J. Sági, A. Szabolcs, A. Szemzö, L. Ötvös, and J. Kypr. 1988. Poly(amino^{2}dA-dT) isomerizes into the unusual X-DNA double helix at physiological conditions inducing Z-DNA in poly(dG-m^{5}dC). *J. Biomol. Struct. Dyn.* 6:503–510.

Vorlíčková, M., P. Sedláček, J. Kypr, and J. Šponar. 1982. Conformational transitions of poly(dA-dT) · poly(dA-dT) in ethanolic solutions. *Nucleic Acids Res.* 10:6969–6979.

Vorlíčková, M., V. Sklenář, and J. Kypr. 1983. Salt-induced conformational transition of poly(dA-dT) · poly(dA-dT). *J. Mol. Biol.* 166:85–92.

Wang, A. H.-J., G. J. Quigley, F. J. Kolpak, G. van der Marel, J. H. van Boom, and A. Rich. 1981. Left-handed double helical DNA: variations in the backbone conformation. *Science.* 211:171–176.

Wells, R. D., J. E. Larson, R. C. Grant, B. E. Shortle, and C. R. Cantor. 1970. Physicochemical studies on polydeoxyribonucleotides containing defined repeating nucleotide sequences. *J. Mol. Biol.* 54:465–497.

Wintero, A. K., M. Fredholm, and P. D. Thomsen. 1992. Variable (dG-dT)$_n$ · (dC-dA)$_n$ sequences in the porcine genome. *Genomics.* 12: 281–288.

Xu, Q., S. R. B. Jampani, and W. H. Braunlin. 1993a. Rotational dynamics of hexaminecobalt(III) bound to oligomeric DNA: correlation with cation-induced structural transitions. *Biochem. USA.* 32:11754–11760.

Xu, Q., R. K. Shoemaker, and W. H. Braunlin. 1993b. Induction of B-A transitions of deoxyoligonucleotides by multivalent cations in dilute aqueous solutions. *Biophys. J.* 65:1039–1049.

Zehfus, M. H., and W. C. Johnson, Jr. 1981. Properties of P-form DNA as revealed by circular dichroism. *Biopolymers.* 20:1589–1603.

Zehfus, M. H., and W. C. Johnson, Jr. 1984. Conformation of P-form DNA. *Biopolymers.* 23:1269–1281.

Zenklusen, J. C., I. Bieche, R. Lidereau, and C. J. Conti. 1994. (dC-dA)$_n$ microsatellite repeat D7S522 is the most commonly deleted region in human primary breast cancer. *Proc. Natl. Acad. Sci. USA.* 91: 12155–12158.

Zhong, L., and W. C. Johnson, Jr. 1990. Poly(dG-m^{5}dC) can assume the Z′ form: a CD study. *Biopolymers.* 30:821–828.