Comparison of the Solution and Crystal Conformations of (G + C)-Rich Fragments of DNA

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ABSTRACT DNA fragments crystallize in an unpredictable manner, and relationships between their crystal and solution conformations still are not known. We have studied, using circular dichroism spectroscopy, solution conformations of (G + C)-rich DNA fragments, the crystal structures of which were solved in the laboratory of one of the present authors. In aqueous trifluorethanol (TFE) solutions, all of the examined oligonucleotides adopted the same type of double helix as in the crystal. Specifically, the dodecamer d(CCCCCGCGGGGG) crystalized as A-DNA and isomerized into A-DNA at high TFE concentrations. On the other hand, the hexamer d(CCGCGG) crystallized in Z-form containing tilted base pairs, and high TFE concentrations cooperatively transformed it into the same Z-form as adopted by the RNA hexamer r(CGCGCG), although d(CCGCGG) could isomerize into Z-DNA in the (NaCl + $NiCl_2$) aqueous solution. The fragments crystallizing as B-DNA remained B-DNA, regardless of the solution conditions, unless they denatured or aggregated. Effects on the oligonucleotide conformation of 2-methyl-2,4-pentanediol and other crystallization agents were also studied. 2-Methyl-2,4-pentanediol induced the same conformational transitions as TFE but, in addition, caused an oligonucleotide condensation that was also promoted by the other crystallization agents. The present results indicate that the crystal double helices of DNA are stable in aqueous TFE rather than aqueous solution.

INTRODUCTION

Knowledge of their molecular structure considerably improves understanding of the biological properties of nucleic acids and proteins. Molecular structures are mostly determined by x-ray diffraction crystallography, which provides the most direct access to their atomic details. However, relevance of the crystal structures to the situation in solution, and to the in vivo environment, still remains uncertain. That is why the present article deals with relationships between the crystal and solution structures of DNA oligonucleotides previously crystallized in the laboratory of one of the present authors (Urpi et al., 1989; Verdaguer et al., 1991; Malinina et al., 1994; Urpi et al., manuscript in preparation). They are (G + C)-rich because duplexes of short (A + T)-rich DNA fragments do not provide suitable crystals (Shakked et al., 1981). In addition, the short (A + T)-rich duplexes are unstable in solution. Besides other aspects, we study effects of 2-methyl-2,4-pentanediol (MPD) on the oligonucleotide conformation in solution because this organic solvent is frequently used as a precipitant for oligonucleotide crystallization. It has recently been shown that MPD diminishes oligonucleotide bending (Sprous et al., 1995), so that DNA probably is even more bent in solution than indicated by the oligonucleotide crystal structures. Maybe there are also other differences between crystal structures of DNA fragments and their conformations in aqueous solution.

The nonalternating (G + C)-rich oligodeoxynucleotides mostly crystallize as A-DNA (for reviews, see Haran and Shakked, 1988; Heinemann et al., 1990), but their solution conformation is B-DNA (Rinkel et al., 1986; Benevides et al., 1986; Wolk et al., 1989; Huber et al., 1991; Fabian et al., 1993). A-form duplex is even adopted by the octamer d(ATGCGCAT) in the crystal state, whereas in solution this sequence is B-type (Clark et al., 1990). The idea has been advocated of an intermediate B/A solution conformer adopted by the nonalternating (G + C)-rich oligodeoxynucleotides (Fairall et al., 1989; Fairall and Finch, 1992; Borden, 1993), which is supported by an observation that oligo(dC)·oligo(dG) unwinds the double helix as compared to the standard B-DNA (Biburger et al., 1994). The apparent discrepancy between the solution and crystal conformations can originate from the fact that the oligonucleotide environments are dramatically different in the crystal and in solution, and this difference can substantially change the oligonucleotide conformation. With this in mind, we study here solution conformations of (G + C)-rich oligonucleotides whose crystal structures are known, and show that the oligonucleotide crystal and solution conformations are closely related if they are compared under comparable environmental conditions.

CD spectroscopy is a method suitable for the extensive mapping of conformational properties of nucleic acids in solution (e.g., Vorlíčková, 1995). However, one should be careful with the CD spectra interpretation, because a single

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CD spectrum can be misleading. For example, the unusual B-form of poly(dI-dC) (Vorlíčková and Sági, 1991) provides a Z-like CD spectrum (Mitsui et al., 1970) although it is not Z-DNA (Sutherland and Griffin, 1983). The CD spectrum of poly(dG-dC) is inverted during the B-Z transition (Pohl and Jovin, 1972), and proflavine binding to poly(rC-rA) also inverts the CD spectrum but not the helix handedness (Westhof and Sundaralingam, 1984). In addition, the Z-like CD spectrum is provided by the A-form of r(CGCGCG) (Uesugi et al., 1984) and by a mitomycin complex with B-form DNA (Tomasz et al., 1983). A further example is poly(dG)·poly(dC) and related DNA molecules exhibiting an A-like CD spectrum, although they are B-DNA in solution according to Raman spectroscopy (Benevides et al., 1986). The danger of erroneous interpretation is minimized by measuring the exhaustive sets of equilibrium CD spectra each DNA sample can provide under various conditions, and analyzing how the CD spectra change from one shape to another. Consequently, the present study is based on an extensive CD spectroscopy mapping of perhaps all conformational states accessible to the analyzed DNA fragments in solution.

MATERIALS AND METHODS

The oligodeoxynucleotides d(CCCCCGCGGGGG), d(CCGCGG), d(CGCTAGCG), and d(GGCGCC) have been prepared as described previously (Urpi et al., 1989; Verdaguer et al., 1991; Malinina et al., 1994; Urpi et al., manuscript in preparation). They were purified by high-performance liquid chromatography, and the purity was better than 99%. The hexamer d(GCGCGC) was bought from Genosys. In a denaturing polyacrylamide gel, this oligonucleotide migrated as a single band with an expected electrophoretic mobility. The RNA hexamer r(CGCGCG) was prepared by the H-phosphonate method according to a modified protocol (Ott et al., 1993), with a coupling time of 3 min, on a 1.5 μmol scale. Cleavage of the crude oligoribonucleotide from the controlled pore glass support and deprotection were accomplished by successive treatment with saturated methanolic ammonia (24 h) and 1 M tetrabutyl ammonium fluoride in dimethylformamide (24 h) at room temperature. Work-up of the deprotection mixture was carried out on a DEAE-Sephacel column (1 × 2 cm), using 0.01 and 1 M LiCl as eluents, followed by desalting on a G-15 Sephadex column (2 × 40 cm). The resulting crude RNA was further purified by 20% polyacrylamide gel electrophoresis in the presence of 7 M urea and extracted from the gel, and the solution was subsequently batched with DEAE-Sephacel in a buffer containing 200 mM sodium chloride, 20 mM sodium acetate, pH 5.2, and 10 mM magnesium chloride. Elution of RNA was achieved by washing the column with a buffer containing 1 M LiCl, 20 mM sodium acetate, pH 5.2, and 10 mM magnesium chloride. The resulting solution was desalted on a G-15 Sephadex column as described above. MPD and spermine were products of Fluka and Serva, respectively.

We took special care with the sample preparation because it follows from our studies (Vorlíčková et al., unpublished data) that CD spectra of some d(C_nG_n) fragments are often irreproducible in the sense that they depend on factors such as the oligonucleotide concentration in the measured sample as well as the oligonucleotide concentration in the stock solution, time since the oligonucleotide dilution, temperature, ionic strength, and pH, even in the course of preparation of the DNA sample. The quadruplex-forming tendency of the DNA fragments, containing runs of G on the 3′ end, may be behind these phenomena. That is why we carefully checked all of these factors with the present oligonucleotides. Yet they did not exhibit the problems described above. All CD spectra shown were measured at equilibrium conditions, i.e., they did not change with time. We have also paid attention to the reversibility and kinetics of the observed CD spectral changes. All described conformational transitions, which did not include DNA aggregation, were reversible.

CD spectra were measured at various DNA concentrations. Most of the measurements were made in 0.1- or 1.0-cm path-length cells, in which the samples can precisely be titrated by MPD and the other agents studied here. In these measurements the concentration of DNA was 1–0.07 mM (in bases), giving the sample UV absorbance within 0.6–0.9. To check the shape of the CD spectrum of d(CCCCCGCGGGGG) in the crystallization solution, we had to use a 0.005-cm path-length sandwich cell to compensate for the high oligonucleotide concentration. All of the cells were bought from Hellma. The CD measurements were made with a Jobin-Yvon Mark IV dichrograph calibrated with isoandrosterone. Oligonucleotide concentrations were determined on the Philips PU-8750 spectrophotometer. Their extinction coefficients are summarized in Table 1. They were calculated from the oligonucleotide hypochromic effects and the molar extinction coefficients of their single strands determined according to the method of Borer (1975).

RESULTS

CD spectroscopic properties of d(CCCCCGCGGGGG)

A duplex of the dodecamer d(CCCCCGCGGGGG) provides the unusual A-like CD spectrum (Fig. 1 *A*) characteristic of most (G + C)-rich DNA molecules starting with cytosine on the 5′-end (Sato et al., 1986; Wolk et al., 1989). The spectrum is dominated by a distinct positive band at 262.5 nm, whose stronger variant is typical for A-DNA (Gray et al., 1972; Ivanov et al., 1974; Gray and Ratliff, 1975; Vorlíčková et al., 1990). Another interesting feature is the negative band at 284 nm (Sato et al., 1986). In a low-salt neutral buffer (used in Fig. 1 *A*), the dodecamer duplex melts in a wide neighborhood of 65°C. The CD spectrum of the single-stranded dodecamer is weak and starts with a positive band at long wavelengths (Fig. 1 *A*), as usual with

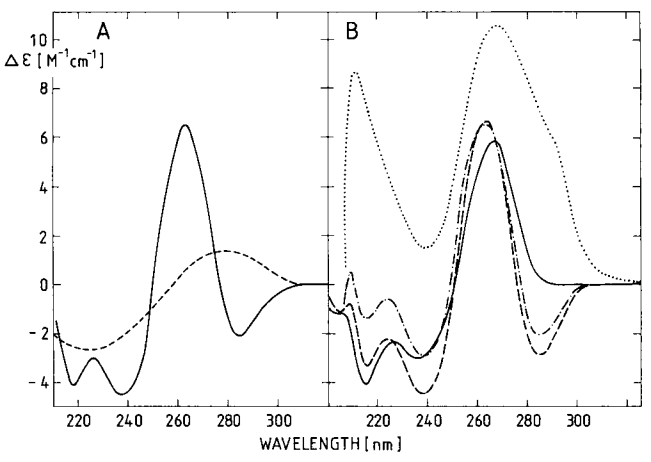

FIGURE 1 CD spectra of d(CCCCCGCGGGGG). (*A*) The measurements were carried out in 1 mM sodium phosphate + 0.3 mM EDTA, pH 6.8, at 20.5°C (——) and 89°C (– – –). (*B*) The measurements were carried out in the presence of the following crystallization agents and their combinations: 30 mM sodium cacodylate, pH 7.1, and 1.25 mM spermine, 19°C (——); 1 mM spermine + 5 mM $CaCl_2$, 19°C (– – –); 1.3 mM spermine + 5 mM $CaCl_2$ + 26.7% (v/v) MPD, 4°C (–·–·–); 1.2 mM spermine + 5 mM $CaCl_2$ + 35.4% MPD, 4°C (·····).

TABLE 1 List of oligonucleotides studied in the present work, their crystallization conditions, extinction coefficients, and crystal and solution structures

Oligonucleotide	Cryst. conditions	Ext. coef. (M^{-1} cm^{-1})	Crystal struct.	Aqueous solution structures	TFE struct.
d(CCCCCGCGGGGG)	30 mM Na cacodylate, pH 7, 5 mM $CaCl_2$, 1.25 mM spermine, 5% (20%)* MPD	8260	A[#]	non-A	A
d(CCGCGG)	10 mM ammonium buffer, 20% (40%)* MPD	8380	tilted Z[§]	B,[¶] Z-DNA**	Z-RNA
d(GGCGCC)	14.3 mM Na cacodylate, pH 7, 21.4 mM $MgCl_2$, 1.4 mM spermine, 2.8 mM Arg acetate, 15% (30%)* MPD	8140	B[¶]	B	B
d(GCGCGC)	—	8220	—	B,Z-DNA**	B
d(CGCTAGCG)	6 mM Na cacodylate, pH 6, 9 mM Ala Arg acetate, 3.75 mM $MgCl_2$, 0.35 mM spermine, 10% (35%)* MPD	9000	B[‖]	B	B
r(CGCGCG)	—	7700	—	A,Z-RNA[##]	A

*Values in parentheses indicate MPD concentrations in the reservoir.
[#]Verdaguer et al. (1991).
[§]Malinina et al. (1994).
[¶]Urpi et al. (1989).
[‖]Urpi et al., manuscript in preparation.
**In ($NaCl+NiCl_2$), Fig. 6.
[##]In ($NaClO_4$+EtOH), Fig. 5.

denatured DNA at high temperatures. This demonstrates that both the strong positive band at 262.5 nm and the small negative band at 285 nm reflect specific properties of the dodecamer native conformation. Its CD spectrum was the same in 0.1-cm and 1.0-cm path-length cells in which the oligonucleotide concentrations differed by a factor of 10. The CD spectrum of d(CCCCCGCGGGGG) was independent of time after dilution from the stock solution and reliably reproducible if we avoided temperatures higher than 20°C at low ionic strength when the dodecamer switched into a foldback. We have also measured dependences of the CD spectrum of d(CCCCCGCGGGGG) on pH and can say that its unusual A-like shape does not originate from cytosine protonation. Nor does it originate from a dodecamer tetraplex formation, although the CD spectrum of parallel-stranded tetraplex is similar (Jin et al., 1992; Lu et al., 1993).

Effects of crystallization conditions on the CD spectrum of d(CCCCCGCGGGGG)

The d(CCCCCGCGGGGG) dodecamer duplex crystallized as A-DNA (Verdaguer et al., 1991). We were interested in what happens with the dodecamer in solution in the presence of crystallization agents, i.e., MPD, spermine, divalent cations, and their combinations (Table 1). At room temperature, the addition of spermine to d(CCCCCGCGGGGG) led to a complete disappearance of the negative long-wavelength band at 285 nm and to a slight depression and a red shift of the positive maximum at 260 nm (Fig. 1 *B*), and the oligonucleotide condensed at low temperatures (not shown). The spermine-induced condensation was promoted by both $MgCl_2$ and MPD, which were used for this oligonucleotide crystallization. Remarkably, 1 mM or higher $CaCl_2$ concentrations eliminated the condensation and restored (Fig. 1 *B*) the CD spectrum recorded in the absence of spermine. This spectrum remained unchanged upon the addition of MPD up to the 5% concentration used for the dodecamer crystallization (Verdaguer et al., 1991), and nothing significant happened with the CD spectrum up to about 30% MPD (Fig. 1 *B*). Thus the dodecamer essentially had the same conformation in the low-salt aqueous and crystallization solutions (Fig. 1 *B*, Table 1). This conclusion also holds for the 24 mM (in bases) dodecamer concentration used for the crystallization. Further increasing the MPD concentration above 30% in 0.1-cm cells caused an abrupt change in the dodecamer CD spectrum (Fig. 1 *B*). A strong positive maximum appeared at 265 nm, which usually accompanies formation of A-DNA, but a strong positive signal, instead of the negative one typical of A-DNA, arose at 210 nm. Together with a slight positive light scattering observed at long wavelengths, these changes indicated an oligonucleotide condensation.

The transition of d(CCCCCGCGGGGG) into A-DNA

Further analysis was performed in ethanol solutions containing no $MgCl_2$ and spermine, that is under conditions under which A-DNA is formed in many natural and synthetic nucleic acids (Ivanov et al., 1974; Vorlíčková et al., 1990, 1991). However, ethanol induced no CD changes, indicating a dodecamer isomerization into A-

DNA. One possible explanation of this observation was that the dodecamer assumed A-DNA even in aqueous solution (Basham et al., 1995). However, the behavior of d(CCCCCGCGGGGG) in aqueous trifluorethanol (TFE) solution, which is a stronger inducer of A-DNA than ethanol (Ivanov et al., 1985; Vorlíčková et al., 1992), disproved this interpretation. TFE induced (Fig. 2) a cooperative transition of d(CCCCCGCGGGGG) with a midpoint at 68%, whereas the resulting CD spectrum was truly typical of A-DNA (Ivanov et al., 1974, 1983; Vorlíčková et al., 1991). It contained not only the very strong band at 269.5 nm (ellipticity of about 12 M^{-1} cm^{-1}), but also the negative band at 213 nm that accompanies A-DNA formation (Ivanov et al., 1985; Vorlíčková et al., 1991, 1992). The transition was reversible and its kinetics was fast. Cooperativity of the TFE-induced transition (Fig. 2, *inset*) reflects the presence of a high-energy barrier between the two isomerizing conformations. This implies that the aqueous conformer of the d(CCCCCGCGGGGG) dodecamer is not A-DNA, despite its A-like CD spectrum.

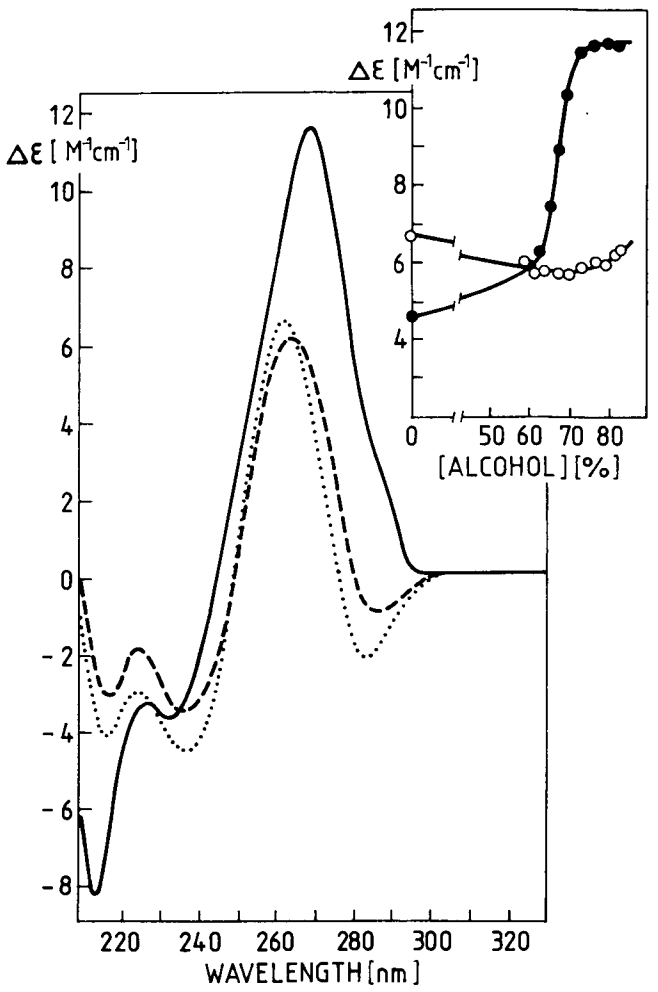

FIGURE 2 CD spectra of d(CCCCCGCGGGGG) in 1 mM sodium phosphate + 0.3 mM EDTA, pH 6.8, at 0.5°C (·····), and in the presence of 80% (v/v) ethanol (– – –) and 75.8% (v/v) TFE (——), which were added as 96% aqueous and 100% solutions, respectively, to the oligonucleotide dissolved in the original low-salt solution. The dependences on the alcohol concentration were measured at −4.5°C. (*Inset*) The dodecamer ellipticity changes induced by (○) ethanol and (●) TFE at 262.5 nm and 269 nm, respectively.

High MPD concentrations also induce A-DNA in d(CCCCCGCGGGGG)

Ethanol and mainly TFE, but not methanol (Malenkov et al., 1975), induce A-DNA conformation in solution, and MPD, used for the oligonucleotide crystallization, had not yet been studied for this effect. Effects of varying concentrations of MPD on the CD spectra of the d(CCCCCGCGGGGG) are shown in Fig. 3. MPD induced a highly cooperative (Fig. 3 *B*) transition of the dodecamer, but only at higher concentrations than TFE. The transition was accompanied by the appearance of a deep negative band at 210 nm, just as in aqueous TFE. The long-wavelength negative band was suppressed, but the strong positive band characteristic for A-forms of most (but not all) DNA molecules did not appear. The same changes in the CD spectra, i.e., the appearance of a deep negative band at 210 nm and only a red shift of the positive band at 260 nm, accompanied the TFE-induced B-A transition of d(CCCCGGGG) (Vorlíčková, unpublished results) and d(CCCGGG) (Wolk et al., 1989). These oligonucleotides are further examples of a correspondence between the crystal and TFE solution structures.

MPD also induced a highly cooperative B-A transition in poly(dA-dT)·poly(dA-dT) (not shown). The CD spectrum of this A-form was similar to that induced by ethanol or TFE at higher than optimum ionic strength. We thus carried out the MPD-induced transition of d(CCCCCGCGGGGG)

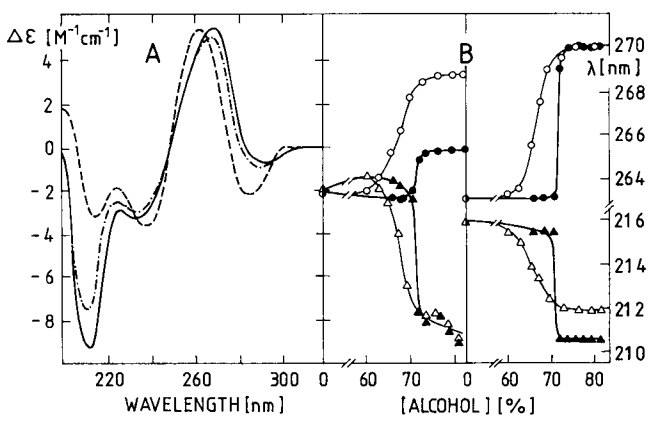

FIGURE 3 Changes in the CD spectrum of d(CCCCCGCGGGGG) caused by MPD that was added to the dodecamer dissolved in 1 mM sodium phosphate + 0.3 mM EDTA, pH 6.8. The measurements were carried out at −8°C. (*A*) The CD spectra in 70.3% (– – –), 72.0% (–·–·–), and 81.2% (——) MPD. (*B*) The transitions of d(CCCCCGCGGGGG) induced by MPD (●, ▲) and TFE (○, △). The transitions were monitored (left) through the ellipticity values at 285 nm (○, ●) and 211 nm (△, ▲), and (right) through the wavelength of the positive long-wavelength ellipticity maximum (○, ●) and negative short-wavelength minimum (△, ▲).

at various ionic strengths and temperatures but always obtained the same CD spectra (Fig. 3), whose positive band ellipticity at 260 nm did not exceed 6 M^{-1} cm^{-1}. The CD spectra had never intersected in isoelliptic points in the course of the transition, so that the transition included more than two different chiral states of the dodecamer. The similarity of the MPD-induced and TFE-induced changes (Fig. 3 *B*) indicated that MPD stabilized the A-form in d(CCCCCGCGGGGG), although the transition was accompanied by another process, most probably oligonucleotide condensation (no UV light scattering was observed, however). In the presence of 0.6 mM spermine, which is a known A-DNA promoter (Minyat et al., 1978), the same transition was observed at a lower, (i.e., 65%) MPD concentration. Thus both MPD and spermine promoted the A-form of d(CCCCCGCGGGGG).

Effects of crystallization agents on d(CCGCGG)

The A-like CD spectrum, although with lower amplitudes, was also exhibited by the hexamer d(CCGCGG) (Fig. 4). However, infrared spectroscopy showed that the hexamer adopted a B-form in aqueous solution (Urpi et al., 1989). The hexamer shares the dodecamer central region but lacks the three base pairs on both ends. Ethanol (Fig. 4 *A*), as well as low spermine and $MgCl_2$ concentrations (Fig. 4 *B*), caused no substantial changes in the CD spectrum of d(CCGCGG). MPD condensed the hexamer in the presence of spermine and $MgCl_2$. In the absence of the other crystallization agents, high MPD concentrations induced an abrupt change in the hexamer CD spectrum, reducing all of its bands above 220 nm and inverting the negative long-wavelength band (Fig. 4 *B*).

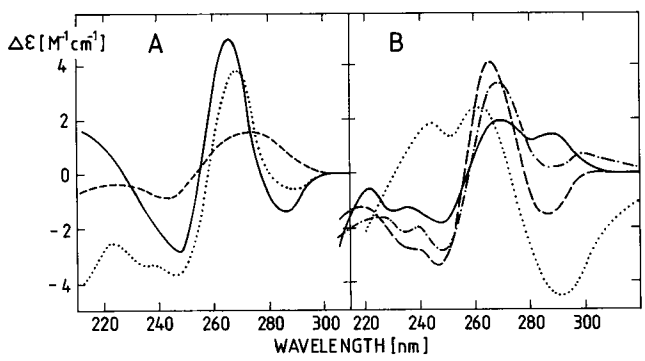

FIGURE 4 CD spectra of d(CCGCGG). (*A*) The measurements were carried out in 1 mM sodium phosphate + 0.3 mM EDTA, pH 6.8, at 1.2°C (——) and 49°C (– – –), and at 0°C (·····) after the addition of ethanol to give a 79.2% concentration. (*B*) The measurements were carried out in the presence of the following crystallization agents and their combinations (at room temperature, except for the last spectrum): 20 mM sodium cacodylate, pH 7, + 5 mM $MgCl_2$ + 1 mM spermine (– – –); 14 mM sodium cacodylate, pH 7, + 5 mM $MgCl_2$ + 0.7 mM spermine + 30.2% MPD (–·–·–); 12 mM sodium cacodylate, pH 7, + 5 mM $MgCl_2$ + 1.7 mM spermine + 38.4% MPD (·····); 0.23 mM sodium phosphate + 0.07 mM EDTA, pH 6.8, + 77% MPD, measured at −3.5°C (——).

Z-RNA conformation of d(CCGCGG)

Better than MPD, high TFE concentrations induced the same cooperative (Fig. 5, *inset A*) and reversible two-state transition of the hexamer, and the resulting conformer was not A-DNA. Its CD spectrum (Fig. 5) contained a positive band at 285 nm, a small maximum at 260 nm, and another maximum at 220 nm followed by a deep negative band at 202 nm. Remarkably, the same CD spectrum is provided by the Z-RNA duplex of r(CGCGCG) (Fig. 5, *inset B*; Hall et al., 1984; Davis et al., 1986). The CD spectrum contains the negative band close to 200 nm characteristic for left-handed DNA duplexes (Riazance et al., 1987). The UV absorption changes (Fig. 5, *inset C*), especially the long-wavelength shoulder on the main absorption band, also indicate the transition of d(CCGCGG) into a Z-form (Pohl and Jovin, 1972). Thus TFE transformed the DNA hexamer d(CCGCGG) into a Z-form with features of Z-RNA rather than Z-DNA. Remarkably, this corresponds to the d(CCGCGG) crystal Z-form, where the base pairs are tilted (Malinina et al., 1994), which is typical for A-RNA and presumably Z-RNA (Trulson et al., 1987) but not Z-DNA (Wang et al., 1979). Thus again, TFE transformed the oligonucleotide into the same type of double helix in solution as observed in the crystal. In addition, MPD pushed d(CCGCGG) toward the same conformation as TFE (Fig. 5, *inset A*). Spermine, which is known to stabilize Z-DNA (Thomas and Messner, 1986), decreased the MPD concentration inducing this transition. The TFE-induced transition of d(CCGCGG) into Z-RNA did not depend on the oligonucleotide concentration (which changed by an order of magnitude) and it was reversible, and its kinetics was fast.

Z-DNA conformation of d(CCGCGG)

We also attempted to induce the hexamer Z-form by the usual combination of NaCl and $NiCl_2$ (Bourtayre et al., 1987) and indeed obtained (Fig. 6 *A*) a CD spectrum typical of Z-DNA and not Z-RNA. Thus d(CCGCGG) can adopt either Z-DNA or Z-RNA, depending on the conditions in solution. Z-DNA of a related alternating hexamer d(GCGCGC) provided an almost identical CD spectrum (Fig. 6 *B*). In addition, these two hexamers isomerized into Z-DNA at nearly identical conditions, suggesting that the terminal bases exerted no influence on their Z-DNA stabilities. In the crystal structure, the terminal base pairs are disrupted, and their bases are swung away from the double helix to pair with the complementary bases of the neighboring oligonucleotide duplex, giving rise to a pairing of the two duplexes (Malinina et al., 1994).

The alternating hexamer d(GCGCGC) provided a CD spectrum typical of B-DNA in low-salt aqueous solution, consisting of the positive and negative bands around 280 and 250 nm, respectively (Fig. 6 *B*). This is a dramatic difference from the CD spectrum of the B-form of d(CCGCGG) (Fig. 4 *A*), differing from d(GCGCGC) only by

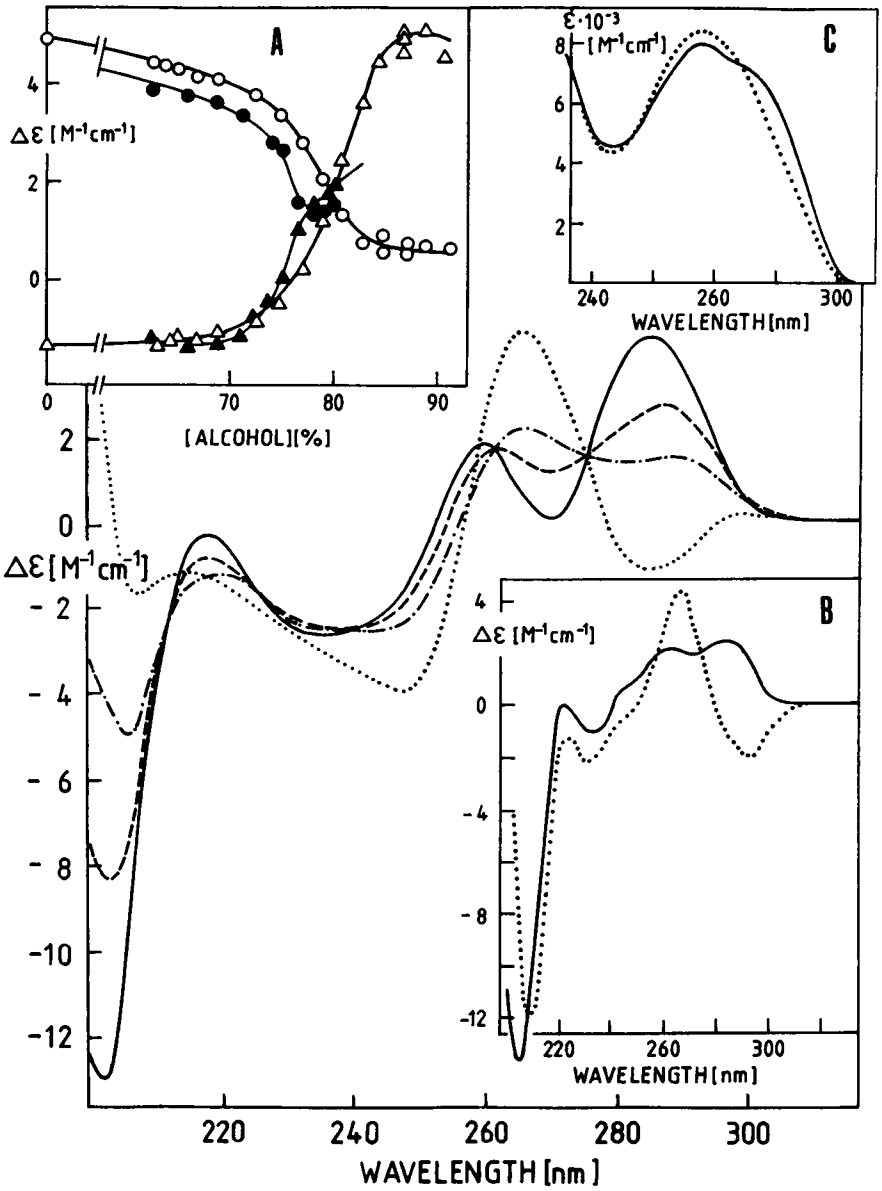

FIGURE 5 TFE-induced changes in the CD spectrum of d(CCGCGG). TFE was added to the oligonucleotide dissolved in 1 mM sodium phosphate + 0.3 mM EDTA, pH 6.8, to give the following concentrations: 63.0% (·····), 79.3% (–·–·–), 81.0% (– – –), and 85.0% (——). Temperature: −2°C. (*Inset A*) The transition of d(CCGCGG) induced by TFE (○, △) and MPD (●, ▲), and monitored by changes in ellipticity at 266 nm (○, ●) and 287.5 nm (△, ▲). (*Inset B*) CD spectra of the A-form and Z-form of r(CGCGCG) in 10 mM sodium phosphate + 0.2 mM EDTA, pH 7.4, 0°C (A-form) (·····), and plus 3.4 M $NaClO_4$ and 31.3% ethanol (Z-RNA) (——). (*Inset C*) UV absorption spectra of d(CCGCGG) in 63.0% (·····) and 85.0% (——) TFE. The alcohol was added to the oligonucleotide dissolved in 1 mM sodium phosphate + 0.3 mM EDTA, pH 6.8. Temperature: −2°C.

an exchange of the terminal bases. Comparison of the CD spectra suggests that, in contrast to the hexamer Z-forms, the low-salt aqueous B-DNA conformers of d(GCGCGC) and d(CCGCGG) differ significantly. This difference is a subject of our further studies.

Constitutive B-forms of d(GGCGCC) and d(CGCTAGCG)

Another crystallized hexamer had the sequence d(GGCGCC) (Urpi et al., 1989). It provided (Fig. 7, *top panels*) a conservative B-type CD spectrum of the same shape as d(GCGCGC) (Fig. 6 B), but with amplitudes as high as that of the positive band of d(CCGCGG) (Fig. 4 *A*). CD spectra of the single-stranded d(CCGCGG) and d(GGCGCC) were almost identical, suggesting that the difference in the CD spectra of their duplexes (compare Fig. 7 *A* and Fig. 4 *A*) reflected their distinct native conformations. The hexamer d(GGCGCC) maintained (Fig. 7 *A*) B-DNA not only at high ethanol but also TFE concentrations that induced the conformational transitions in d(CCCCCGCGGGGG) and d(CCGCGG). The amplitudes of the CD spectra were small in the alcohols, as usual with B-DNA. TFE concentrations higher than 76% aggregated d(GGCGCC). The crystallization agents also had no effect unless the B-form of this oligonucleotide aggregated (Fig. 7 *B*). We did not succeed in destabilizing this B-form under any conditions. It could only denature. This hexamer crystallized as B-DNA (Urpi et al., 1989). The octamer d(CGCTAGCG) behaved in a similar way (Fig. 7, *bottom panels*). Its B-form CD spectrum resembled that of d(GGCGCC) (Fig. 7 *A*), in spite of their quite different primary structures. Again, the octamer remained B-DNA (or aggregated) in both ethanol and TFE (Fig. 7 *C*) and in the presence of the crystallization agents or

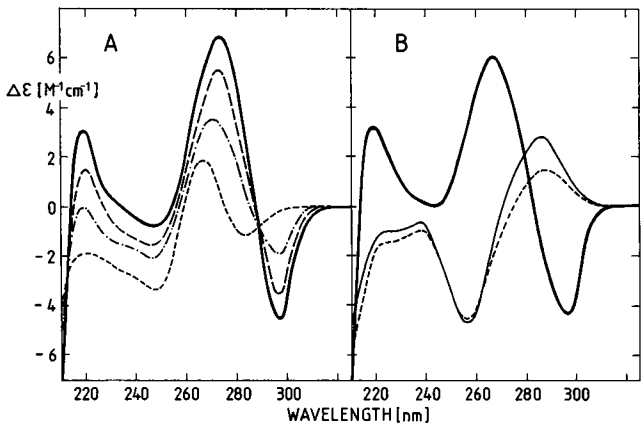

FIGURE 6 CD spectra of (*A*) d(CCGCGG) and (*B*) d(GCGCGC) measured in 10 mM Tris-HCl, pH 7.5, at 23°C. (*A*) 5 M NaCl (– – –); 5 M NaCl + the following $NiCl_2$ concentrations: 3 mM (–·–·–), 4.8 mM (— —), 9.6 mM (——). (*B*) 0 M NaCl (——), 5 M NaCl (– – –), 5 M NaCl + 4.8 mM $NiCl_2$ (——).

their combinations (Fig. 7 *D*). Nor did NaCl + $NiCl_2$ destabilize its B-form. The oligonucleotide aggregated above 20 mM $NiCl_2$. It also provided a B-DNA crystal (Urpi et al., manuscript in preparation).

DISCUSSION

This paper describes CD and UV absorption spectroscopy studies in solution of the conformational behavior of (G + C)-rich DNA fragments whose crystal structures have been solved (Urpi et al., 1989; Verdaguer et al., 1991; Malinina et al., 1994; Urpi et al., manuscript in preparation). The oligonucleotides were studied under various conditions in solution, including those known to induce non-B duplexes, to 1) assess the effects of MPD and other crystallization agents on their conformation, and 2) analyze relationships between their crystal and solution conformations.

The first conclusion following from the present analysis is that conformational polymorphism of the (G + C)-rich DNA fragments in solution includes the conformation in which the DNA fragments crystallize. The DNA fragments that adopt only the B-form regardless of the solution conditions and otherwise only denature or aggregate, crystallize in the B-form. On the other hand, the oligonucleotides adopting non-B duplexes in the crystals are B-form under most conditions in solution, but they switch into the non-B crystal duplex in about 75% aqueous trifluorethanol. This non-B crystal duplex is A-DNA in the case of d(CCCCCGCGGGGG) and Z-form with d(CCGCGG), whereas the A-form is not adopted by d(CCGCGG) and the Z-form is not adopted by d(CCCCCGCGGGGG). The correlation between the crystal and TFE conformations even extends to the conformational details of the Z-form because TFE induces Z-RNA in d(CCGCGG), rather than Z-DNA, although Z-DNA is also accessible to d(CCGCGG) in solution, whereas the oligonucleotide crystal Z-form contains unusually tilted base pairs, which presumably occur in Z-RNA (Trulson et al., 1987) but not Z-DNA (Wang et al., 1979). Second, MPD is shown here to induce the same conformational transitions in the (G + C)-rich DNA fragments as TFE. Similar effects of MPD and TFE have also been observed with calmodulin (Bayley and Martin, 1992), in which both agents increased the α-helix content. The conformational transitions induced by MPD are, however, very close to the oligonucleotide condensation necessary for crystallization. Spermine helps to induce the non-B conformations and condense the oligonucleotides. However, the concentrations of MPD used for crystal growing are much lower than those that induce oligonucleotide isomerization in solution. Thus, the oligonucleotides should only isomerize into the crystal non-B conformations during the crystallization process, and no transition takes place as long as they are dissolved in the crystallization solution. Instead of the A-DNA preferred by d(CCCCCGCGGGGG), d(CCGCGG) adopts a Z-form in both TFE and MPD solutions and in the crystal. This difference from d(CCCCCGCGGGGG) originates from the dominance of A-DNA-promoting CC and GG steps in the dodecamer (Minchenkova et al., 1986), whereas the Z-DNA-promoting CG and GC steps dominate in the hexamer (Peticolas et al., 1988). It is not clear what the conformation of the terminal cytosine in the hexamer Z-form is in solution, but the crystal and solution Z-forms even seem to share the unusual base pair tilting because the CD spectrum of d(CCGCGG) corresponds to Z-RNA rather than Z-DNA at high TFE concentrations.

The present results indicate that the crystal double helix type of (G + C)-rich oligonucleotides of DNA can be predicted, taking advantage of the CD spectral changes induced by TFE. This relatively straightforward approach could be used to choose sequences that are likely to crystallize in a predetermined type of double helix, before the time-consuming hit-or-miss oligonucleotide crystallization process is started (Ho et al., 1991). Further tests of this possibility are in progress in our laboratory.

The present observations document that the crystal structures are highly relevant to the oligonucleotide conformations in solution. However, the crystal structures may correspond to the oligonucleotide conformations adopted in the presence of about 75% aqueous TFE rather than in aqueous solution, where oligonucleotides can fold into radically different conformers. DNA is certainly hydrated much less in cell nuclei than in dilute aqueous solutions, so that it is not at all clear that DNA conformations stabilized by TFE are irrelevant to the situation in vivo.

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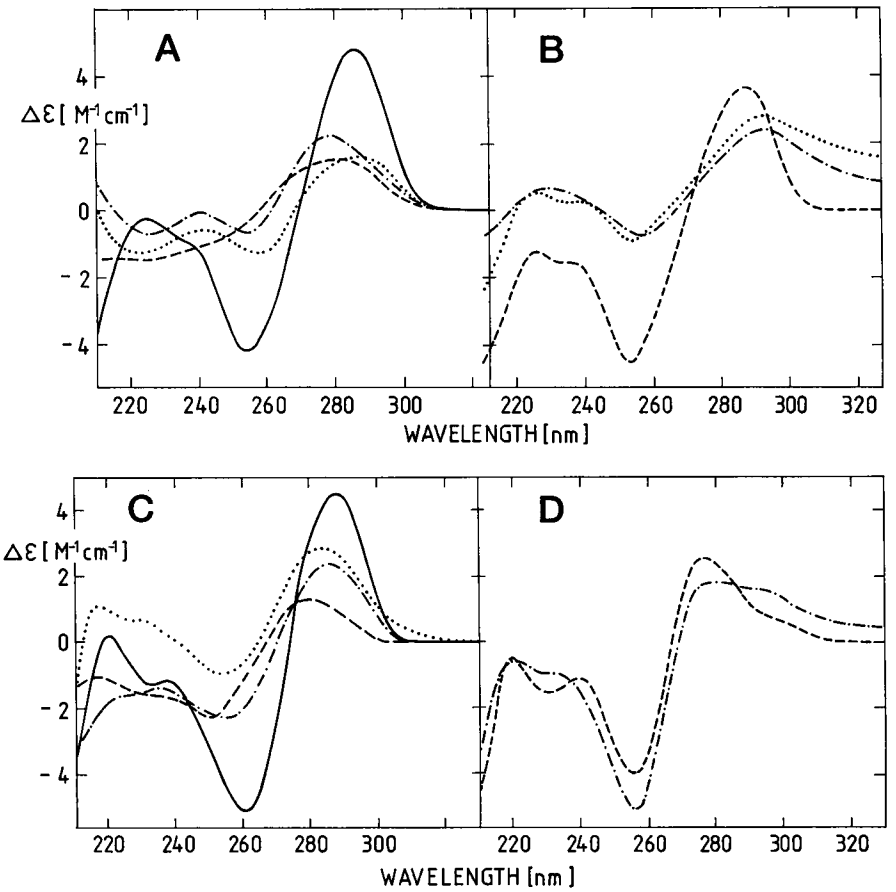

FIGURE 7 CD spectra of d(GGCGCC) (*A* and *B*) and d(CGCTAGCG) (*C* and *D*). (*A* and *C*) The measurements were carried out in 1 mM sodium phosphate, 0.3 mM EDTA, pH 6.8, at (*A*) 0.7°C (——), 52.5°C (– – –), and at 0°C in the presence of 79.5% ethanol (·····) or 75.4% TFE (–·–·–); (*C*) 0.5°C (——), 75.0°C (– – –), and at 0°C in the presence of 81.9% ethanol (·····) or 78.6% TFE (–·–·–). (*B* and *D*) The CD spectra were measured in 20 mM sodium cacodylate, pH 7, plus 5 mM $MgCl_2$ and 1 mM spermine (– – –), and in the presence of (*B*) ethanol added to give a 5% concentration (·····), or (*B* and *D*) MPD added to give a 5% concentration (–·–·–). Temperature: 0°C.

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