3466–3473 *Nucleic Acids Research, 1999, Vol. 27, No. 17*

Aqueous trifluorethanol solutions simulate the environment of DNA in the crystalline state

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Received May 24, 1999; Revised and Accepted July 21, 1999

ABSTRACT

We took 28 fragments of DNA whose crystal structures were known and used CD spectroscopy to search for conditions stabilising the crystal structures in solution. All 28 fragments switched into their crystal structures in 60–80% aqueous trifluorethanol (TFE) to indicate that the crystals affected the conformation of DNA like the concentrated TFE. The fragments crystallising in the B-form also underwent cooperative TFE-induced changes that took place within the wide family of B-form structures, suggesting that the aqueous and crystal B-forms differed as well. Spermine and magnesium or calcium cations, which were contained in the crystallisation buffers, promoted or suppressed the TFE-induced changes of several fragments to indicate that the crystallisation agents can decide which of the possible structures is adopted by the DNA fragment in the crystal.

INTRODUCTION

X-ray diffraction is the major source of information about the molecular structure of DNA. However, DNA should be crystallised in order to undergo X-ray diffraction analysis but the crystallisation solutions of nucleic acid fragments frequently contain alcohols, cations and other agents (1) that change the conformation of DNA (2). In addition, the process of crystallisation makes DNA aggregate, which may also influence its conformation (3). Hence the relationship between the crystal and solution structures is not straightforward.

Studies of particular DNA fragments (4–8), an RNA–DNA hybrid (9), a ribozyme (10) and a self-complementary RNA dodecamer (11) have shown that their solution structures differed from those adopted in the crystalline state. The differences included not only different types of double helix, but also significant differences in the double helix bending (12–18) and winding (18,19). The average solution B-DNA has a decreased helical twist, positive roll and negative slide compared with the majority of high resolution crystal B-DNA structures (19). Even the apparently sequence-dependent variations in DNA are rather a consequence of environment in the crystalline state (20). Hence the crystalline environment evidently influences the conformation of nucleic acids. Here we describe conditions in solution simulating the DNA environment in the crystalline state.

MATERIALS AND METHODS

The DNA fragments used in this work were synthesised, purified and characterised as described previously (21). Their nucleotide sequences, crystal structures and the crystallisation conditions (22–48) are summarised in Table 1. The lyophilised DNA fragments were dissolved in 1 mM Na phosphate, 0.1 mM EDTA, pH 7. Their concentrations were determined from their UV absorption spectra measured at 25°C in the above buffer using the Unicam 5625 UV/Vis spectrometer and the molar extinction coefficients given in Table 1. The coefficients were determined according to Gray (49) while the hypochromic effects of the oligonucleotides were taken into account.

CD spectra were measured using the Jobin-Yvon Mark IV and VI spectrometers in 0.1 and 0.2 cm pathlength cells (Hellma) placed in a thermostatted holder. The ellipticities are given in $M^{-1}cm^{-1}$, the molarity, M, being related to the nucleoside residues of DNA. The measurements in trifluorethanol (TFE, Sigma) were mostly taken at 0°C using the Haake DC3 cryostat under the conditions detailed in the figure legends.

RESULTS

We first describe the relevant conformational properties of 13 DNA fragments crystallising in the A-form. They all were found to undergo a two-state, cooperative transition induced by 60–80% TFE. The transitions and the resulting CD spectra of 10 DNA fragments are summarised in Figure 1. The CD spectra share the deep negative band around 210 nm, a negative shoulder in the vicinity of 230 nm and the strong positive band between 260 and 270 nm. These spectral features are characteristic for the A-form (50). Minor differences between the CD spectra of the particular DNA fragments originate from the different CD spectroscopic properties of the particular dinucleotides (51) and from the sequence-dependent variations of the A-form (52). The cooperative nature of the transitions demonstrates that neither fragment adopted the crystal structure, i.e. the A-form, in the aqueous buffer. All of them switched into the crystal structure only in 60–80% TFE. Consequently, the aqueous TFE simulates the DNA environment in the crystals. Two spectra of d(GCCGGC) are given in Figure 1 to demonstrate that CD spectral features of the A-form of this hexamer were enhanced

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Miriam died in a car accident in May 1998 at the age of 28. This article is dedicated to her memory.

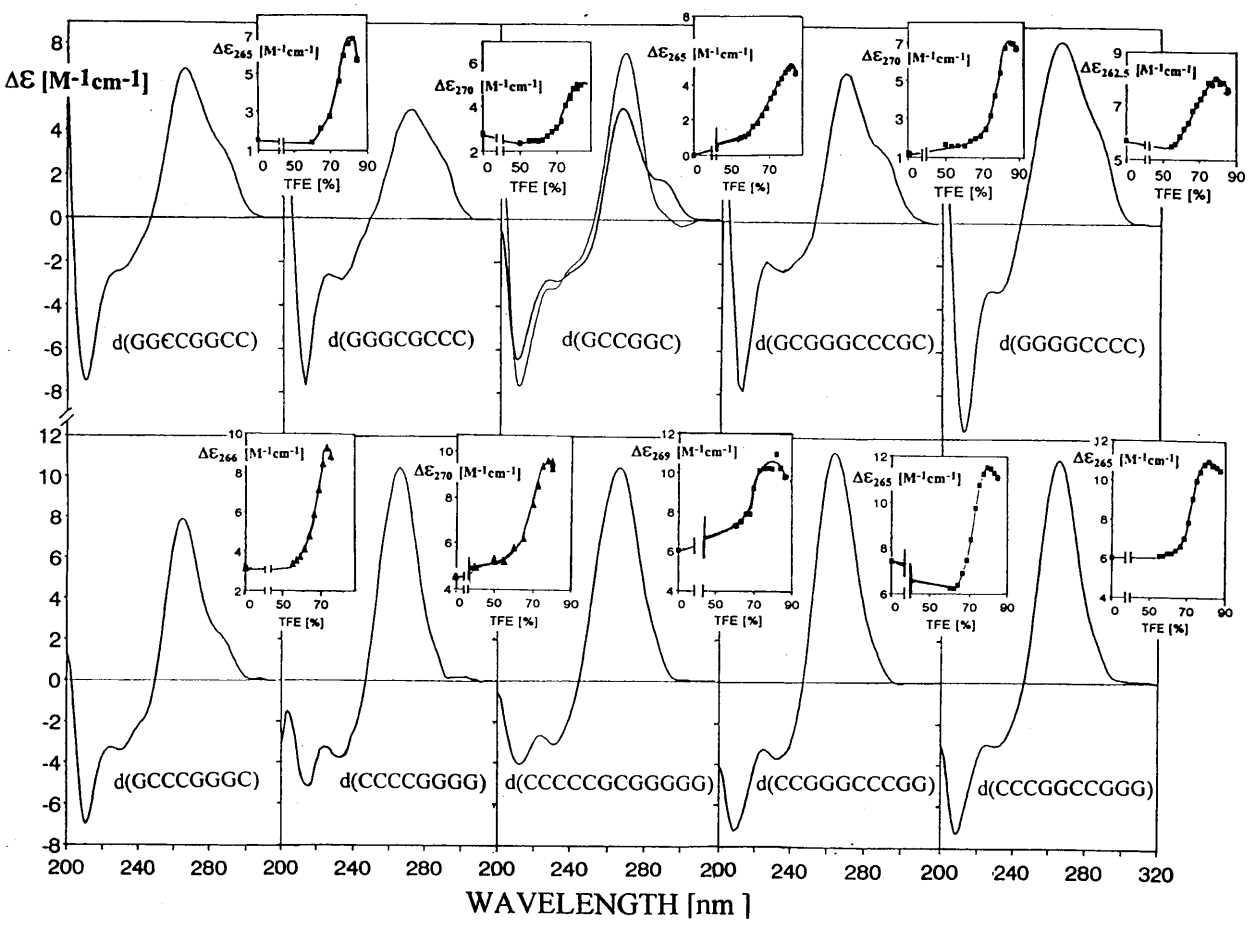

Figure 1. CD spectra of the TFE-induced A-forms of the indicated (G+C) oligonucleotides of DNA crystallising in the A-form. Inserts: dependencies on the concentration of TFE of the oligonucleotide ellipticities at the wavelength of the positive maximum. TFE was added stepwise to the oligonucleotides dissolved in (squares) 1–2 mM Na phosphate, 0.3–0.6 mM EDTA, pH 7, or in (triangles) 10 mM Na phosphate, 0.3 mM EDTA, pH 7. The spectra were measured within 0–3°C in 2 mm pathlength cells. The A-forms were measured in the following solutions. d(GGCCGGCC): 82.5% TFE, 0.4 mM Na phosphate, 0.1 mM EDTA; d(GGGCGCCC): 81% TFE, 0.4 mM Na phosphate, 0.1 mM EDTA; d(GCCGGC) (bold trace): 81.5% TFE, 0.3 mM Na phosphate, 0.05 mM EDTA; d(GCCGGC) (light trace): the same conditions plus 0.2 mM $MgCl_2$; d(GCGGGCCCGC): 82.1% TFE, 0.2 mM Na phosphate, 0.06 mM EDTA; d(GGGGCCCC): 79.1% TFE, 0.2 mM Na phosphate, 0.06 mM EDTA; d(GCCCGGGC): 66.3% TFE, 0.7 mM Na phosphate, 0.5 mM EDTA; d(CCCCGGGG): 80% TFE, 2 mM Na phosphate, 0.007 mM EDTA; d(CCCCCGCGGGGG): 75.2% TFE, 0.2 mM Na phosphate, 0.07 mM EDTA; d(CCGGGCCCGG): 77.4% TFE, 0.5 mM Na phosphate, 0.08 mM EDTA; d(CCCGGCCGGG): 81.1% TFE, 0.2 mM Na phosphate, 0.06 mM EDTA.

upon the addition of magnesium cations which were present in the crystallisation solution (24). This enhancement was surprising because divalent magnesium cations are known to destabilise the A-form of other DNA molecules (53).

The 10 DNA fragments summarised in Figure 1 were composed of G and C only, making their duplexes sufficiently thermostable for the solution studies in aqueous TFE. The three remaining present DNA fragments, crystallising in the A-form, contained the destabilising AT pairs. The decamer d(ACCCGCGGGT) contained 20% AT pairs that did not hinder the TFE-induced transition into the A-form even at low ionic strength (Fig. 2). Yet the amplitudes of its A-form diagnostic CD bands were larger at the higher ionic strength (Fig. 2, left insert). The dodecamer d(GCGTACGTACGC) contained 33% AT pairs. It failed to isomerise into the A-form at low ionic strength. The ionic strength increase and temperature lowering were needed for TFE to switch this fragment into the A-form (Fig. 2). This A-form was also induced at low ionic strength, but only after the addition of hexaminecobalt (not shown) that was present in the dodecamer crystallisation solution (Table 1). The duplex of the 13th fragment, i.e. d(GGTATACC), was still less thermostable owing to its 50% A+T content. Yet the octamer switched into the A-form if the ionic strength was increased and temperature simultaneously lowered (Fig. 2). This A-form was even induced at low ionic strength in the presence of barium and spermine (Fig. 2, right panel, insert b) which were both contained in the octamer crystallisation solution (Table 1).

We have also explored conformational behaviour in solutions of DNA fragments longer than four nucleotides which crystallised in the Z-form (Table 1). Figure 3 shows CD spectra of four of them in aqueous TFE. The spectra share a weak negative

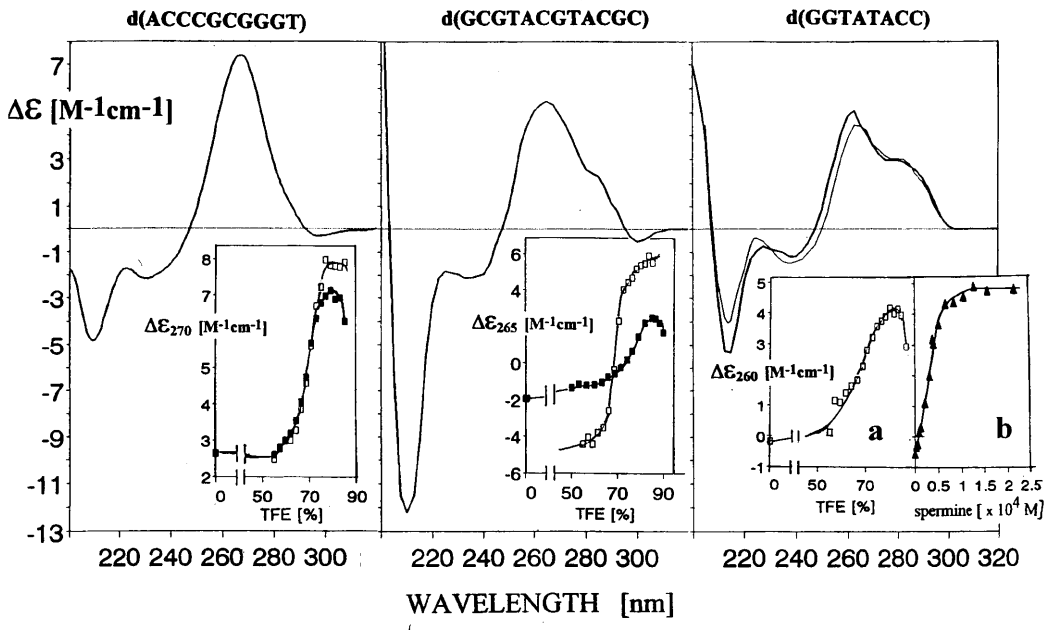

Figure 2. CD spectra of the A-forms of: (left) d(ACCCGCGGGT) in 77% TFE, 0.2 mM Na phosphate, 0.07 mM EDTA; (middle) d(GCGTACGTACGC) in 82% TFE, 2 mM Na phosphate, 1 mM EDTA; and (right) d(GGTATACC) in (light trace) 82% TFE, 2 mM Na phosphate, 0.05 mM EDTA, or in (bold trace) 75% TFE, 0.2 mM $BaCl_2$, 0.13 mM spermine, 0.4 mM Na phosphate, 0.002 mM EDTA. Inserts: ellipticity dependencies on the concentration of TFE added to the indicated oligonucleotides dissolved in: (left, closed squares) 1 mM Na phosphate, 0.3 mM EDTA, (left, open squares) 10 mM Na phosphate, 0.3 mM EDTA; (middle, closed squares) 2 mM Na phosphate, 0.6 mM EDTA, (middle, open squares) 10 mM Na phosphate, 0.3 mM EDTA; (right, a) 10 mM Na phosphate, 0.3 mM EDTA, (right, b) spermine-induced CD changes in 75% TFE, 0.3 mM Na phosphate, 0.05 mM EDTA and 0.2 mM $BaCl_2$. All buffers were at pH 7. The measurements were performed at 0°C.

band at 290 nm, a positive band at 260 nm, another small positive band at 215 nm and, particularly, the deep negative band at 200 nm. The cooperative nature of the TFE-induced transition, as well as the characteristic CD spectral features (50), tend to favour the B–Z transition. Hence these four fragments also obeyed the rule that the crystal structures of DNA were adopted in 60–80% TFE. Fragments containing A and T and crystallising in the Z-form were much more difficult to work with in solution. The hexamer d(TGCGCA) remained B-form even in aqueous TFE at low ionic strength (not shown). However, hexaminecobalt, which was present in the crystallisation solution (Table 1), induced the B–Z transition of the hexamer even at low concentrations of TFE (Fig. 4). The decamer d(CGTACG-TACG) required traces of spermine, i.e. the decamer crystallisation agent (Table 1), to switch into the Z-form in which it crystallised. Traces of hexaminecobalt, i.e. another agent used for this oligonucleotide crystallisation, also stabilised the Z-form, but it simultaneously aggregated the oligonucleotide.

Figure 5 shows CD spectra of four DNA fragments crystallising in the B-form. It is remarkable that all four displayed a cooperative TFE-induced change though the CD spectrum retained features typical of the B-form, i.e. the positive band at 275–280 nm and the negative band of a similar magnitude at 245–250 nm. This lack of transition into a non-B conformer was not due to an insufficient duplex thermostability because essentially the same results were obtained in 10 mM Na phosphate (e.g. Fig. 5, far left insert). Neither did temperature lowering to –14°C change the results. The Dickerson dodecamer also displayed a cooperative TFE-induced transition between two B-forms.

Another kind of behaviour was observed (Fig. 6) with d(CCAACGTTGG) and d(CTCTCGAGAG), both crystallising as B-forms (Table 1). They remained B-forms even in TFE at low ionic strength, but changes in their CD spectra indicated a partial transition towards the A-form. If the ionic strength was increased, then d(CCAACGTTGG) switched into the A-form (Fig. 6), though it crystallised in the B-form. However, addition of traces of the calcium cations, which were present in the crystallisation solution (Table 1), inhibited the B–A transition of d(CCAACGTTGG). So the calcium cations were entirely responsible for the correspondence between the crystal and aqueous TFE structure of this oligonucleotide. A similar behaviour was exhibited by d(CTCTCGAGAG) which also isomerised into the A-form in aqueous TFE if the ionic strength was sufficiently high. However, it also contained divalent calcium cations in the crystallisation buffer (Table 1) which hindered the transition in solution (Fig. 6) so that the oligonucleotide retained B-form both in the crystal and in the aqueous TFE. Another couple of oligonucleotides, i.e. d(CCGGCGCCGG) and d(CATGGCCATG), crystallised as B-forms, but underwent the TFE-induced B–A transition even at low ionic strength (Fig. 7). However, they were reluctant to leave the B-form in the presence of divalent cations in the crystallisation buffers (Table 1), i.e. magnesium with d(CCGGCGCCGG) and calcium with d(CATGGCCATG) (Fig. 7).

Table 1. Nucleotide sequences of the DNA fragments studied in this work, their spectroscopic properties[a], crystal structures, references to the articles where the crystal structures and the crystallization conditions were first described, and their conformations in aqueous TFE

DNA fragment primary structure	Molar extinction coefficient ($M^{-1}cm^{-1}$)	Crystal structure	Ref.	Crystallisation agents[b]	Conformation in TFE in the presence of low salt[c]	high salt[d]	cryst agent[e]
GGCCGGCC	8870	A	22	Mg, Spm, MPD	A		
GGGCGCCC	8330	A	23	Mg, Spm, MPD	A	A	
GCCGGC	8570	A	24	Mg, Spm, MPD	A	A	A (Mg)
GCGGGCCCGC	8420	A	20	Spm, MPD	A		
GGGGCCCC	8690	A	25	Mg	A		
GCCCGGGC	8330	A	26	Mg, MPD or Isop	A	A	A (Mg), agg[f]
CCCCGGGG	9100	A	27	Mg, Spm, MPD	A	A	
CCCCCGCGGGGG	8650	A	28	Ca, Spm, MPD	A		
CCGGGCCCGG	8610	A	29	Mg, Spm, MPD	A	A	
CCCGGCCGGG	8910	A	30	Spm, MPD	A		
ACCCGCGGGT	9000	A	31	Ba, MPD	A		
GCGTACGTACGC	8440	A	32	HCo, Spm, Prop	B	A	A (HCo)
GGTATACC	10 040	A	33	Ba, Spm, MPD	B	A	A (Ba+Spm)
CGCGCG	8690	Z	34	Mg, Spm, Isop	Z		
CGCGCGCG	11 200	Z	35	Mg, MPD	Z		
GCGCGCGCGC	8640	Z	36	Mg, Spm, MPD	Z		
GCGCGCG	9170	Z	37	Spm, Prop	Z		
TGCGCA	9480	Z	38	HCo	B		Z (HCo)
CGTACGTACG	8830	Z	39	Spm, HCo, Prop	B		Z (Spm)
GGCGCC	8710	B	40	Mg, Spm, MPD	B	B	
CGCTAGCG	9000	B	41	Mg, Spm, MPD	B		
CGATCGATCG	8610	B	42	Mg, MPD	B	agg[f]	
CGCGAATTCGCG	9210	B	43	Mg, Spm, MPD	B	B, agg[f]	
CCAACGTTGG	8920	B	44	Mg, MPD	B	A	B (Ca)
CTCTCGAGAG	9180	B	45	Ca, MPD	B	A	B (Ca)
CCGGCGCCGG	8100	B	46	Mg, MPD	A	A	B (Mg)
CATGGCCATG	8530	B	47	Ca, Spm, MPD	A		B (Ca)
CCGCGG[g]	9220	Z	48	Spm, Arg, MPD	Z		Z (Spm)

[a]At the absorption maximum which occurred between 253 and 260 nm with all present fragments.
[b]Spm, spermine; Arg, tetra-arginine; Hco, hexaminecobalt; Prop, propanol; and Isop, isopropanol.
[c]1–2 mM Na phosphate + 0.3 mM EDTA, pH 7, diluted by the added TFE.
[d]10 mM Na phosphate + 0.3 mM EDTA, pH 7, diluted by the added TFE.
[e]A(Mg), oligonucleotide A-form is induced in the presence of magnesium cations in the TFE solution.
[f]Oligonucleotide aggregates.
[g]This hexamer has been previously studied (2).

DISCUSSION

The conformation of DNA non-negligibly depends on the environment, which is a long-known fact (53–59) that has so far not been properly taken into account in the extrapolations of the crystal structures of DNA to the situation in solution. It is not even known to what environment DNA is exposed in crystal and whether this environment can be simulated in solution. This is the problem which we have tried to solve in this article. For this purpose, we chose 28 fragments of DNA whose crystal structures were known and measured their CD spectra under various solution conditions which induced conformational transitions of DNA (50).

Naturally, the conditions had to include various concentrations of TFE because it induces the A- and Z-forms of DNA (50). However, the major motivation to use TFE stemmed from our previous demonstration (2) that it induced essentially the same

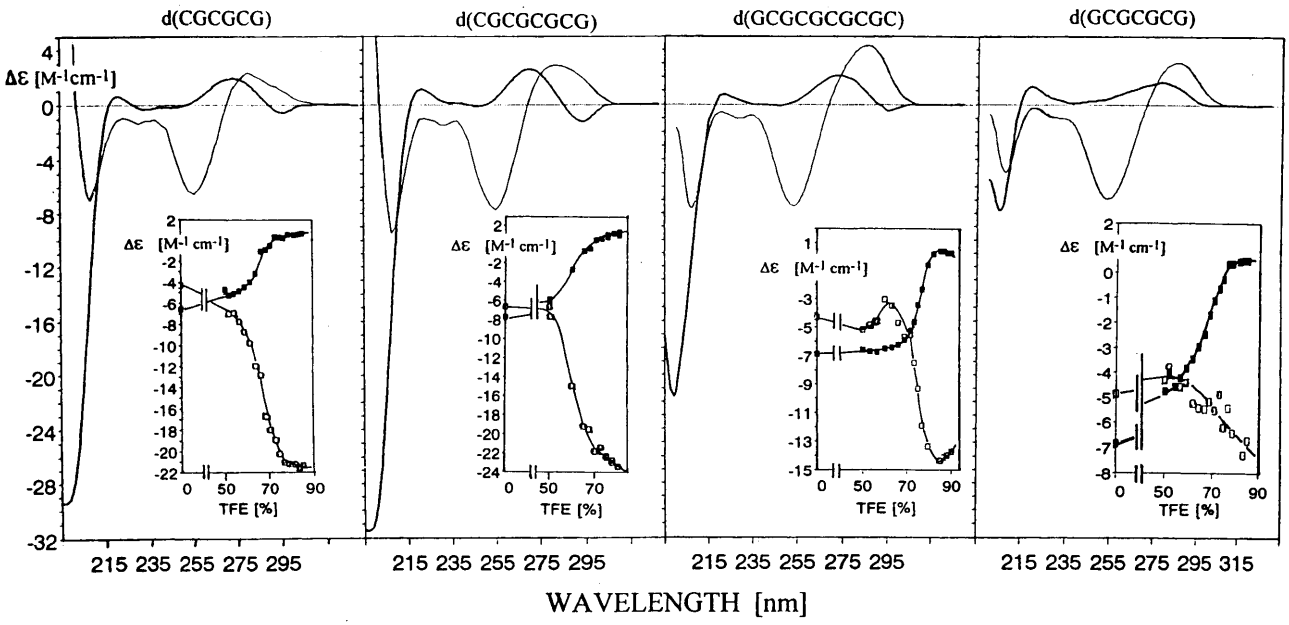

Figure 3. CD spectra of (light traces) B-forms and (bold traces) Z-forms of d(CGCGCG), d(CGCGCGCG), d(GCGCGCGCGC) and d(GCGCGCG). All the B-forms were measured in 1 mM Na phosphate, 0.1 mM EDTA, pH 7, in 0.1 cm cells. The Z-forms were measured after the addition of TFE up to concentrations of 76.8, 70.0, 82.1 and 83.5%, respectively, in 0.2 cm cells. All of the spectra were measured at 0°C. Inserts: TFE-induced ellipticity changes at (closed squares) 255 nm and (open squares) 205 nm.

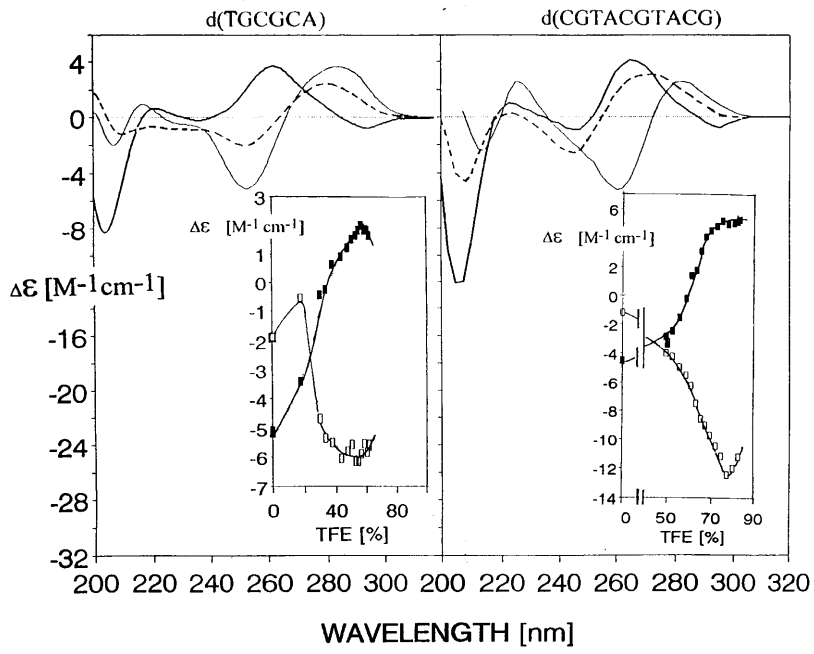

Figure 4. CD spectra of: d(TGCGCA) B-form (light trace) in 0% TFE, 1 mM Na phosphate, 0.1 mM EDTA, (dashed trace) 62% TFE, 0.15 mM Na phosphate, 0.04 mM EDTA and (bold trace) Z-form in 54.7% TFE, 0.004 mM hexaminecobalt, 0.2 mM Na phosphate, 0.06 mM EDTA; d(CGTACGTACG) B-form (light trace) in 0% TFE, 1 mM Na phosphate, 0.1 mM EDTA, (dashed trace) 82% TFE, 0.2 mM Na phosphate, 0.06 mM EDTA and (bold trace) Z-form in 67.3% TFE, 0.03 mM spermine, 0.6 mM Na phosphate, 0.2 mM EDTA. Temperature 0°C, buffer pH was 7. Inserts: TFE-induced ellipticity changes at (closed symbols) 252.5 nm and (open symbols) 208 nm. Both oligonucleotides were dissolved in 2 mM Na phosphate and 0.6 mM EDTA. The solutions of d(TGCGCA) and d(CGTACGTACG) contained a constant 0.004 mM concentration of hexaminecobalt and a constant 0.03 mM concentration of spermine, respectively.

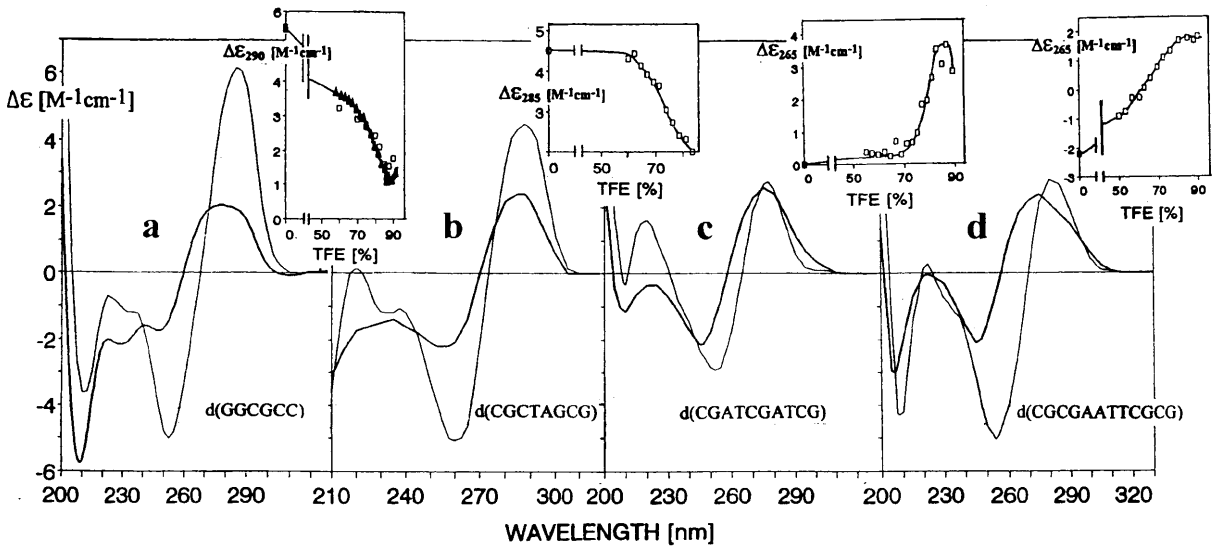

Figure 5. CD spectra of d(GGCGCC), d(CGCTAGCG), d(CGATCGATCG) and d(CGCGAATTCGCG) in (light traces) 0% TFE + (**a**, **b**) 1 mM Na phosphate, 0.3 mM EDTA, pH 7, or (**c**, **d**), 2 mM Na phosphate, 0.6 mM EDTA, pH 7, and (bold traces) the spectra upon the addition of TFE giving the (a) 85, (b) 78.6, (c) 88.3 and (d) 85% concentrations. All of the spectra and dependences were measured at 0°C. Inserts: TFE-induced changes in ellipticity at the indicated wavelengths. TFE was added to the oligonucleotides dissolved in (open squares) 1 or 2 mM Na phosphate and 0.3 or 0.6 mM EDTA, or (closed triangles) in 10 mM Na phosphate, 0.3 mM EDTA, pH 7.

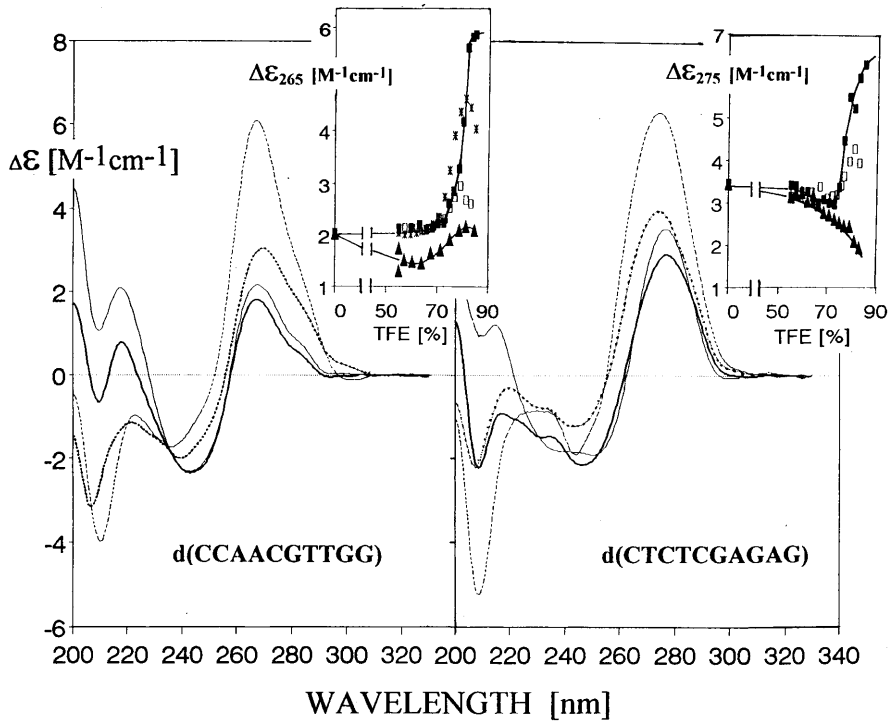

Figure 6. CD spectra of d(CCAACGTTGG) and d(CTCTCGAGAG) in (light traces) 1 mM Na phosphate, 0.1 mM EDTA, pH 7, in the absence of TFE, after the addition of TFE giving the concentrations (dotted traces) 81.2% (0.2 mM Na phosphate, 0.06 mM EDTA), (dashed traces) 85% TFE (1.5 mM Na phosphate, 0.05 mM EDTA), (bold traces): (left) 75.2 TFE, 0.055 mM $CaCl_2$ (0.8 mM Na phosphate, 0.03 mM EDTA); (right) 77.5% TFE, 0.3 mM $CaCl_2$, (0.2 mM Na phosphate, 0.05 mM EDTA). Temperature 0°C. Inserts: TFE-induced ellipticity changes at the indicated wavelengths. TFE was added to d(CCAACGTTGG) in (open squares) 1 mM, (asterisks) 3 mM, or (closed squares) 10 mM Na phosphate + 0.05 mM EDTA, pH 7, (triangles) 3 mM Na phosphate at a constant 0.055 mM concentration of $CaCl_2$. TFE was added to d(CTCTCGAGAG) in 0.3 mM EDTA + (open squares) 1 mM, (closed squares) 10 mM Na phosphate, pH 7, and (triangles) in 10 mM Na phosphate, 0.3 mM EDTA, pH 7, at a constant 0.4 mM concentration of $CaCl_2$.

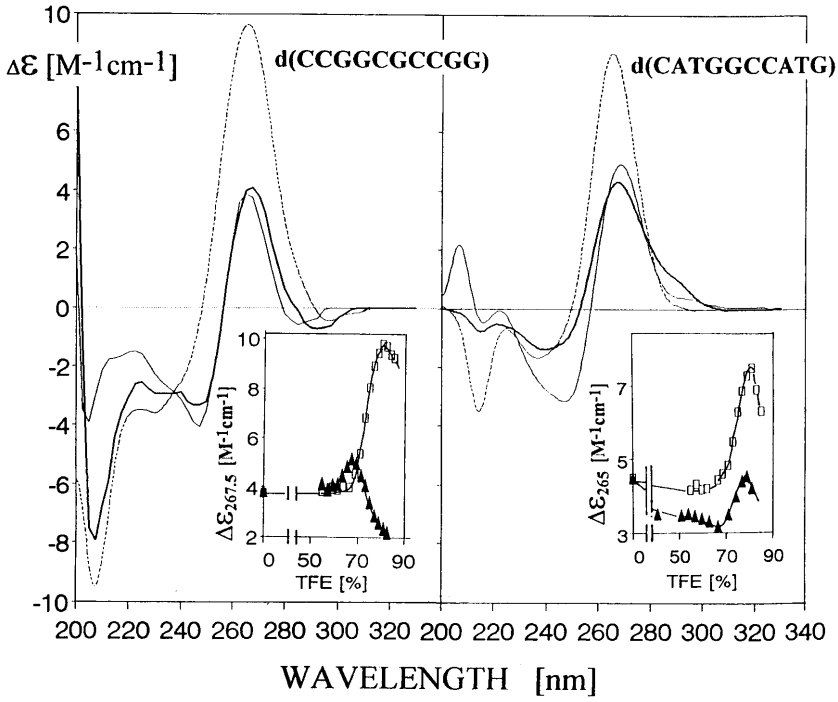

Figure 7. CD spectra of d(CCGGCGCCGG) and d(CATGGCCATG) in (light traces) 1 mM Na phosphate, 0.1 mM EDTA, pH 7, in the absence of TFE, (left) (dashed trace) 82% TFE, 0.2 mM Na phosphate, 0.05 mM EDTA, (bold trace) 73% TFE, 0.2 mM $MgCl_2$, 0.3 mM Na phosphate, 0.005 mM EDTA; (right) (dashed trace) 81% TFE, 0.2 mM Na phosphate, 0.04 mM EDTA, (bold trace) 81% TFE, 0.2 mM $CaCl_2$, 0.2 mM Na phosphate, 0.004 mM EDTA. Inserts: TFE-induced ellipticity changes at the indicated wavelengths. TFE was added to the oligonucleotides in (squares) 1 mM Na phosphate, 0.3 mM EDTA, pH 7, or (triangles) (left) 10 mM Na phosphate, 0.002 mM EDTA, pH 7, at a constant 0.2 mM concentration of $MgCl_2$, and in (right) 1 mM Na phosphate, 0.02 mM EDTA, pH 7, at a constant 0.2 mM concentration of $CaCl_2$.

changes in DNA conformation as MPD, an alcohol frequently used for DNA fragment crystallisation (Table 1). However, work with MPD is difficult in solution because it causes DNA aggregation which hampers the spectroscopic measurements. Hence we used TFE here and were surprised to get the 100% correspondence between the crystal structures and conformations in aqueous TFE with our test sample of 28 DNA fragments.

With a majority of the analysed 28 DNA fragments, correspondence with the crystal structure was obtained in 60–80% TFE containing a little monovalent salt. However, it was necessary to have traces of the crystallisation agents in the aqueous TFE solution to get correspondence with the remaining four oligonucleotides. It was remarkable that the crystallisation agents hindered the TFE-induced conformational transition to a non-B structure in some cases while they exhibited a promotive effect in another cases. Crystallisation of molecules as large as the DNA fragments is very difficult to predict (60). The present study suggests an approach to the DNA crystal structure prediction based on CD spectroscopy measurements in aqueous TFE containing various crystallisation agents. CD spectroscopy works nicely in this simulating solution and can be used to examine a large number of DNA fragments and conditions quite easily in a relatively short time.

We have only studied DNA fragments here but RNA is also crystallised using MPD, spermine and divalent cations (61) so we would anticipate that the crystal and solution structures of RNA would differ significantly as well. Indeed, there are examples reported in the literature (9–11) showing that RNA crystal structures are inconsistent with the results of the solution studies. We suspect that the inconsistencies originate for the same reason as with DNA, i.e. because the relevant RNA molecules adopt different conformations in the aqueous solution than in the crystals. Comparison of the crystal and solution structures of RNA is in progress (62). The α-helical content of calmodulin is increased in solution under conditions favouring protein crystallisation, i.e. in the presence of MPD (63). In contrast, ribonuclease structure is not influenced by MPD (64). Various DNA fragments have also been studied in MPD solutions (2,12–15,65,66).

The fact that particular DNA fragments crystallising in A- or Z-form, are B-form in aqueous solution has been documented by several laboratories (4–8). Here this conclusion is confirmed and generalised on the basis of a systematic study that furthermore shows that even the B-forms are different in the aqueous and TFE solutions. We observe cooperative switching induced by TFE and suspect that the dehydrated, i.e. the TFE-stabilised B-form, is the solution counterpart of the crystal B-form while the aqueous B-form is not. This interpretation of our results is consistent with NMR studies (18,19) showing that the aqueous solution B-forms are underwound and otherwise modified compared with the crystal B-forms.

The present study is also closely relevant to molecular dynamics simulations of DNA (67) which use the crystal structures as the starting or reference data. The present results show that

the realistic MD simulations in water should not reproduce the crystal structures of DNA because the crystal structures are stabilised by alcohol molecules and by other crystallisation agents which are mostly absent in the MD simulations.

Dickerson *et al*. have recently discussed how much DNA is subject to the tyranny of the lattice in the crystals (68). The present work suggests that it is also subject to the tyranny of the crystallisation agents. These almost always include an alcohol and divalent, trivalent or tetravalent cations whose combined effect can change the conformation of DNA substantially.

ACKNOWLEDGEMENT

This work was supported by grant A4004701/1997 awarded to M.V. by the Grant Agency of the Academy of Sciences of the Czech Republic.

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