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NMR, CD and IR spectroscopies of a tridecanucleotide containing a no-base residue: coexistence of B and Z conformations

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ABSTRACT

The synthesis of the tridecadeoxynucleotide d(CGm^{5}CGCGxACATGT), where x is the 1-cyano-2-deoxy-β-D-erythropentofuranose, is described. The NMR, IR, CD studies at various salt concentrations and temperatures of this oligomer show that the B and Z conformations are simultaneously present in the same short DNA fragment. A single apurinic residue is sufficent for the coexistence of the B and Z helices on this oligomer.

INTRODUCTION

After CD studies, left-handed Z-DNA was demonstrated by X-ray cristallographic diffraction of the hexamer CGCGCG [1]. Subsequent studies with different oligonucleotides by other physical chemical methods produced considerable information regarding the interconversion right-to-left handed helix. The Z-DNA is a higher energy conformation which is favored by alterning purine-pyrimidine sequences, chemical modification of the bases, negative supercoiling and other various factors [2]. Immunofluorescence detection of Z-DNA structures using antibodies specific for Z-DNA in chromosomes [3-5] recombinant plasmids, restriction fragments and natural DNAs [6-8] under physiological conditions suggest biological roles for Z-DNA, probably through specific interactions with Z-DNA binding proteins [9].

The existence of left-handed conformations within a long right-handed DNA-helix necessitates the formation of two B-Z junctions. At the present time the exact nature of the B-Z junction is not clearly understood and no spectroscopic evidence with a synthetic model oligonucleotide is available.

The most obvious structural characteristics of a B-Z junction suggest an unwinding of the duplex within a limited length in order to facilitate the transition right to left conformation. Biological proof of the unwinding arises from the sensitivity of B-Z junctions to single strand specific nucleases S1 and Bal-31 [10,11]. The low estimate of the junction's dimensions is 4

to 8 bp with the inhibition of Bam HI cleavage experiments [12] in supercoiled plasmids.

Another possible means to release the torsional stress within a constrained duplex is to remove one base-pair. Molecular models show that a single apurinic (or apyrimidinic) site allows enough flexibility for the B-Z transition. Apurinic sites in DNA may occur from spontaneous depurination [13], exposure to alkylating agents [14,15] or from the action of various DNA glycosylases [16]. When a no-base residue is incorporated into complementary 15-mer strands, this leads to considerable destabilisation of the duplex as measured by the reduction of the melting temperature [17]. In order to test the hypothesis that an apurinic site can provide a flexible joint for two oligonucleotides of different conformations, we decided to synthetize the 13-mer CGm^{5}CGCGxACATGT 1, where x is a no-base residue and to study its conformation at various salt concentrations and temperatures with NMR, IR and CD spectroscopies [18].

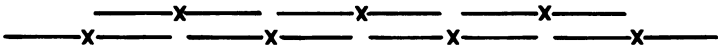
The main characteristics of this model oligonucleotide are the following: 1) the 1-cyano-2-deoxy-β-D-ribose is a stable derivative of 2-deoxyribose and should avoid any accidental cleavage of the 3'-5' phosphate chain by β elimination during the synthesis and purification steps. 2) Hexamers a and b are self-complementary and the 13-mer should form in solution concatamers [19] (Scheme I). 3) The presence of 5-methyl 2'-deoxy cytidine m^{5}C in the a hexamer enables the facile transition into the Z conformation [20-22] at low Na^+ concentrations while leaving the b part in the B conformation [23]. 4) The complete assignment by NMR spectroscopy of the two hexamers was already done [22,23] and their melting temperatures measured.

This paper presents spectroscopic evidences of the simultaneous B and Z conformations on the synthetic 13-mer in presence of sodium counterions.

EXPERIMENTALS PROCEDURES

1-cyano-2-deoxy-β-D-ribose (x) was obtained by complete detoluylation with aqueous 2N NaOH of 1-cyano-3,5-di-O-p-toluyl 2-deoxy-β-D-erythropentofuranose [24], was 5-O-protected with 4,4-dimethoxytrityl chloride and 3-O-phospho rylated with published procedures [25].

The 13-mer was synthetized in solution with the phosphotriester method according to the Scheme II (65 mg, 16 moles). The deprotected oligonucleotide was purified by preparative HPLC (Zorbax ODS 9.3 mm), exchanged to the ammonium form on Dowex 50W NH_4 and lyophilized. The purity was checked on an HS-5 C-18 analytical column and by gel sizing electrophoresis.



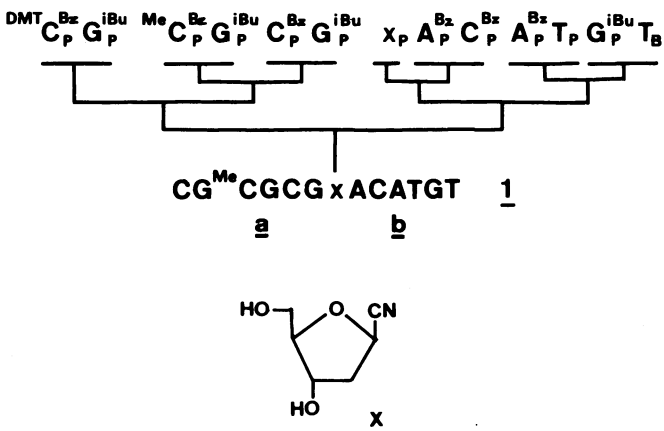
Scheme I

NMR spectroscopy.

The pure oligomer was dissolved in ^2H_2O containing 0.1 M NaCl + 5 mM PO_4^{2-} and was free of possible divalent ions by adding EDTA ($\sim$0.1 mM). The pH was adjusted to 7-8 by the addition of a small amount of NaOH. The sample was lyophilized twice in ^2H_2O and redissolved in ^2H_2O to a final concentration of 2 mM. The solution was introduced into a NMR tube which was then degassed in a vacuum line and sealed. ^{1}H-NMR 500 MHz non exchangeable proton spectra were recorded on a Brucker WM 500 and referenced relative to internal 3-(trimethylsilyl)$^2H_4$ propionic acid (TMP). Two dimensional NOE (NOESY) spectra were recorded using the pulse sequence [90°-t1-90°-τm-90°-t2] [26,27] and the phase cycling described by States [28]. τm was randomly varied by 15% around the average value of 400 ms. A 4-s recycle delay was allowed between each scan and the solvent peak was irradiated during the preparation and evolution periods only. A total of 512 FIDs, 2048 data points each, was recorded. After zero filling in the t1 dimension, a 1024 x 1024 data points matrix was obtained and then Fourier transformed in both dimensions. The 36.5 MHz phosphorus spectra were recorded on a Bruker WH-90 and are referenced relative to an internal standard of trimethyl phosphate.

CD spectroscopy

The tridecamer was dissolved in a 10 mM tris buffer pH = 7.5 in the presence of known concentrations of NaCl and $NiCl_2$. The CD spectra were recor-



Scheme II

ded at 10°C on an Autodichrograph Mark V (Jovin Yvon).

IR spectroscopy

The samples were obtained by dialysing the samples used for CD studies against a NaCl solution so as to obtain 2 NaCl in excess per nucleotide. The pH was adjusted at 7.5 using NaOH. Then the solutions were gently dried on infrared ZnSe transparent supports to obtain homogeneous films. The hydration of the films was monitored by the relative humidity present in a sealed cell in a range 15% to 100% (H_2O or ^2H_2O). The IR spectra of the film were recorded on a Perkin Elmer 180 spectrometer. Digital data issued from the ratiometer was directly transferred to a Hewlett-Packard 9825 A computer allowing data treatement such as base line and water contribution corrections.

RESULTS

^{1}H-NMR studies

(1) Base proton assignment

Figure 1 shows the 500 MHz spectrum of d(CGm^{5}CGCGxACATGT) base protons at 69°C. Also shown in this figure is the spectral region where the apurinic residue H1' proton signal is located and easily identified by its triplet structure. The lack of base deshielding effect explains the low chemical shift value (δ = 4.5 ppm) of this proton compared to those of the other H1' proton signals (between 6.4 and 6.0 ppm). Base proton assignment was performed i) by comparing at high temperature (t > 80°C) the 13-mer spectrum with those of the corresponding hexamers, d(CGm^{5}CGCG) [23] and d(ACATGT) [22] ii) by recording a 2D-NOESY spectrum in order to study the intra- and inter-base NOEs; this experiment was performed at 45°C where the signals of the three methyl group protons (those of the methylated cytidine and of the two thymidines) are well resolved.

Figure 2 shows the region of the 2D-NOESY contour plot containing the cross peaks which connect base proton signals of either the same or adjacent residues. Interactions between CH_3 and H6 protons of the same methylated pyrimidine residue as well as cross peaks corresponding to NOE between CH_3 protons and H8 proton of adjacent purine residues are observed. As previously observed in double helical structures of alternated purine-pyrimidine oligomers [29-31] the H8 purine protons are much closer to the CH_3 protons of the following methylated pyrimidine than to the CH_3 protons of the preceding one (with respect to the 5' 3' direction).

(2) Proton chemical shifts at high temperature

Base proton chemical shifts of the 13-mer measured at 90°C are listed in

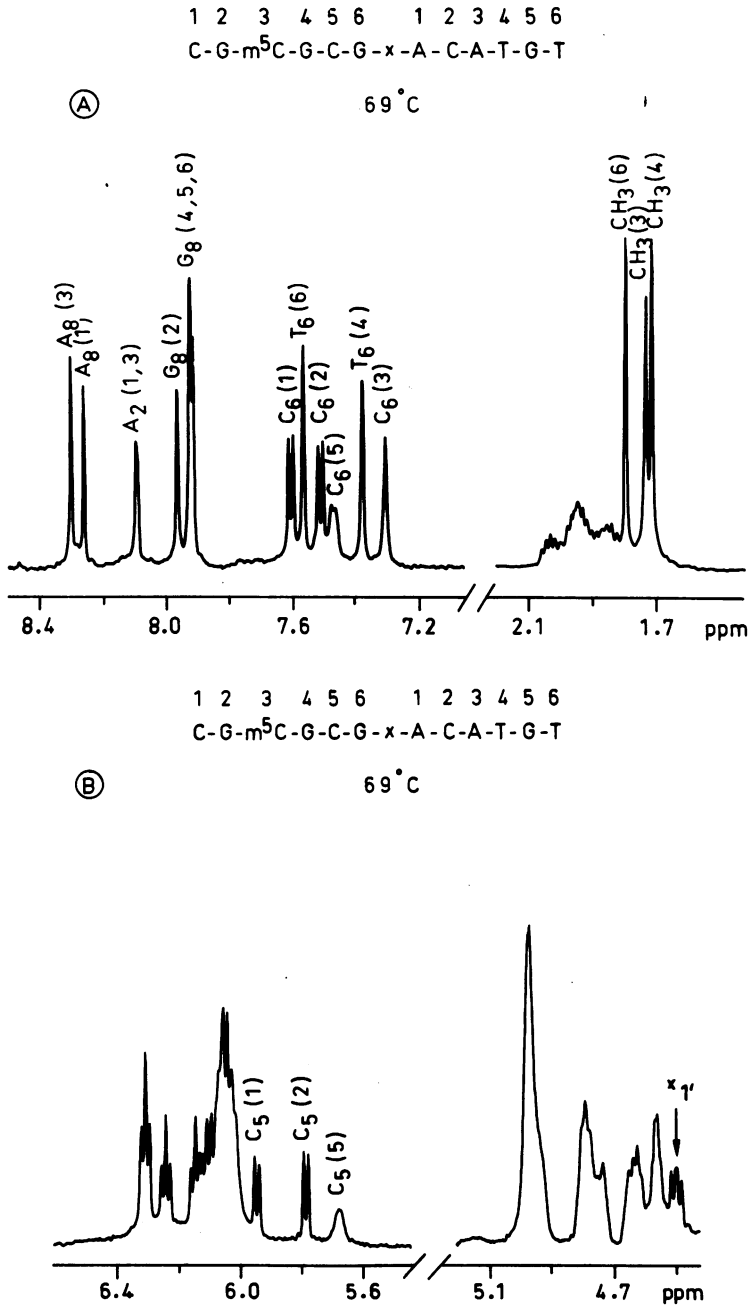


Fig. 1 500 MHz ^{1}H-NMR base proton spectrum of d(CGm^{5}CGCGxACATGT) in aqueous solution (2 mM, 0.1 M NaCl + 5 mM PO_4 =) recorded at 69°C.

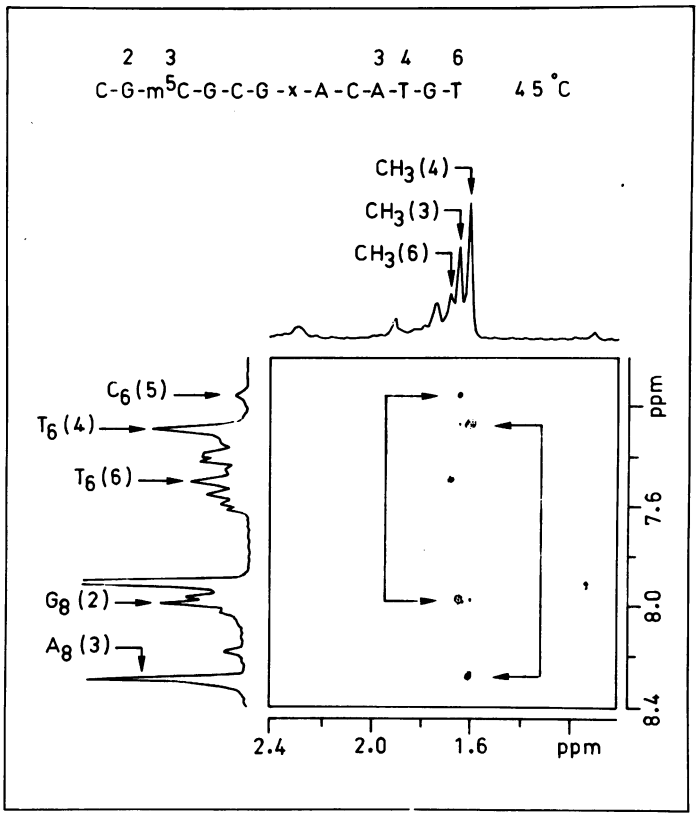


Fig. 2 Part of 2D-NOESY 500 MHz spectrum (contour plot) of d(CGm^{5}CGCGxACATGT) recorded at 45°C containing the intra (H6-CH_3) and inter (CH_3-H8) NOE interactions.

Table I and compared to those of the corresponding protons of d(CGm^{5}CGCG) and d(ACATGT) in the single stranded form (at t = 90°C and t = 75°C respectively) [22,23]. In general, the differences in chemical shift between 13-mer and hexamer protons are slight: the most significant variations involve the two residues surrounding the apyrimidic residue (dC, dG on the left; dA, dC on the right with respect to the 5'→3' direction); these variations are in the range 0.03 to 0.07 ppm.

(3) Temperature dependence of proton chemical shifts. Duplex formation of d(CGm^{5}CGCGxACATGT). On lowering the temperature, the base proton signals of the 13-mer are shifted to higher field with various amplitudes depending on the residue whereas new resonances appear when t° is less than 50°C. These new resonances are poorly resolved in the aromatic region because of their low

Table I: base proton chemical shifts of: (A) d(CGm^{5}CGCGxACATGT); (B) d(CGm^{5}CGCG) [22] and (ACATGT) [23] measured at high (90°C) and low (25°C) temperatures

residue / proton	C	G	m^{5}C	G	C	G
H6 A	7.607	7.954	7.398	7.922	7.607	7.922
H8 B	7.621	7.951	7.419	7.919	7.678	7.951
coil form (90°C)	-0.014	0.003	-0.021	0.003	-0.071	-0.029
H5 A	5.969		1.800		5.831	
H2/CH3 B	5.972		1,797		5.878	
	-0.003		0.003		-0.047	
H6 A	7.560	7.970	7.100	7.870	7.220	7.870
H8 B	7.682	8.012	7.163	7.940	7.363	7.960
B helix (25°C)	-0.122	-0.042	-0.063	-0.070	-0.143	-0.090
H5 A	5.900			1.630		5.350
H2/CH3 B	5.952			1.672		5.469
	-0.052			- 0.042		-0.119

residue / proton	A	C	A	T	G	T
H6 A	8.284	7.560	8.300	7.398	7.922	7.560
H8 B	8.250	7.600	8.303	7.380	7.932	7.572
coil form (90°C)	0.034	-0.040	-0.003	-0.010	-0.010	-0.012
H5 A	8.125	5.830	8.125	1.726		1.800
H2/CH3 B	8.170	5.860	8.110	1.708		1.790
	-0.045	-0.030	0.015	0.018		0.010
H6 A	8.090	7.34	8.237	7.222	7.870	7.130
H8 B	8.220	7.51	8.369	7.167	7.935	7.350
B helix (25°C)	-0.130	-0.170	-0.132	0.055	-0.065	-0.220
H5 A	a)	5.470	a)	1.500		1.320
H2/CH3 B	8.000	5.530	7.820	1.508		1.520
		-0.060		-0.008		-0.200

a) undetected

intensities, the large number of signals and their increasing linewidths but a new resonance is clearly observable in the methyl proton region (Fig. 3). This additional resonance is located at higher field with respect to the three CH_3 signals of methylated pyrimidines and its intensity increases on lowering the temperature suggesting the occurence of a slow exchange process

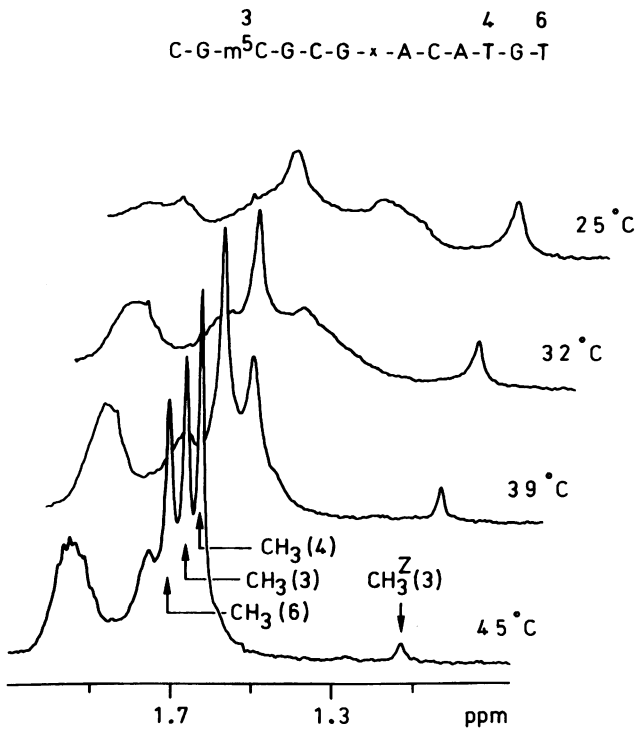


Fig. 3 500 MHz ^{1}H-NMR CH_3 proton spectra of d(CGm^{5}CGCGxACATGT) in aqueous solution (2 mM, 0.1 M NaCl + 5 mM PO_4 =) recorded at different temperatures between 45 and 25°C.

(at the NMR time scale). It is now well-known that 2D-NOESY experiments provide useful information on slowly exchanging species: the longitudinal magnetizations of two signals corresponding to the same proton in two exchanging forms are coupled together and off-diagonal cross connecting these signals can be observed in a 2D-NOESY spectrum [32,33]. Figure 4 shows the (1.0-2.2) ppm part of the 13-mer 2D-NOESY spectrum recorded at 45°C.

A cross peak connecting the additional resonance to the d(m^{5}C) CH_3 signal is observed whereas no interactions involving the CH_3 signals relative to the two thymidines are detected.

Figure 5 plots the chemical shift variations versus temperature of the major signals of the 13-mer base protons and that of the additional CH_3 resonance. The curves relative to the major resonances display a sigmoidal form (when the variations are sufficiently significant) and are very similar to the $\delta = f(t°)$ curves obtained for the B helix-coil transition of numerous

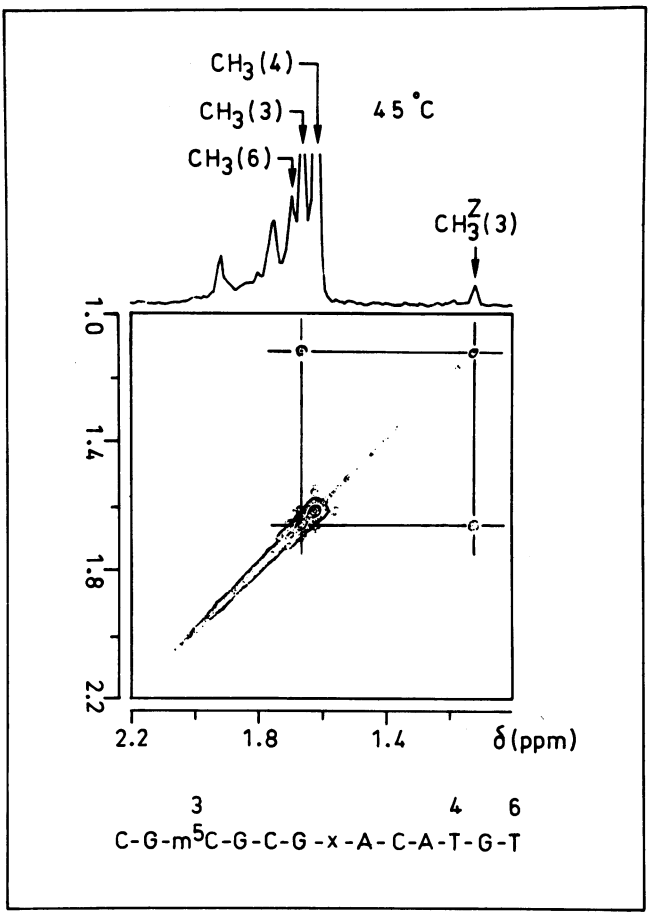


Fig. 4 (1.0-2.2) ppm region of 2D-NOESY 500 MHz spectrum (contour plot) of d(CGm^{5}CGCGxACATGT) recorded at 45°C containing the dm^{5}C CH_3 (B) and CH_3 (Z like) NOE interactions via chemical exchange.

DNA fragments including d(CGm^{5}CGCG) and d(ACATGT) [20-23,29-31,34]. From these melting curves, the midpoint temperature of the B helix-coil transition of the tridecanucleotide can be determined. The obtained values are 62° ± 1°C and 54° ± 2°C for the central residues of d(CGm^{5}CGCG) and d(ACATGT) parts of the sequence respectively. For the corresponding hexamers, the t 1/2 values were 75° and 43°C respectively [22,23].

As mentioned above the 13-mer fragment is only 50% self-complementary and the base pairing can lead to the formation of dimers - one via the d(ACATGT) block, another via the d(CGm^{5}CGCG) block - and/or to the formation of an

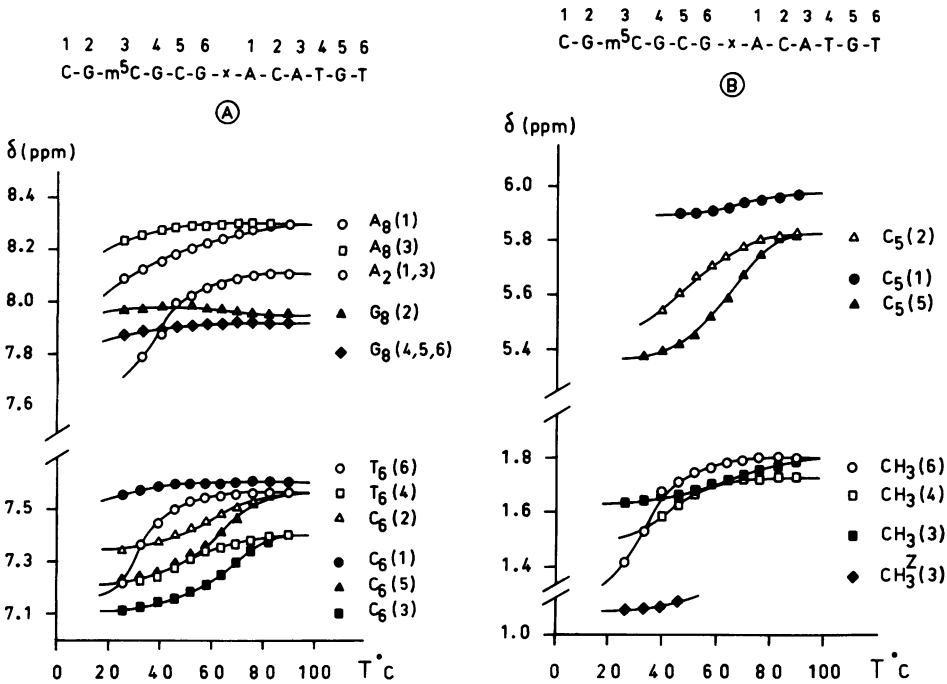


Fig. 5 Temperature dependence od d(CGm^{5}CGCGxACATGT) base proton chemical shifts in 0.1 M NaCl solution.

ordered concatemer as described in the Scheme I. The following arguments indicate the occurence of a coil to concatemer transition : i) the sigmoidal form of the melting curves obtained from the chemical shifts of the major resonances (Fig. 5) reflects a two states transition where the high temperature state is a coil form ii) at low temperature the linewidths of the 13-mer proton signals are much larger than those usually observed for the self-complementary oligonucleotides but similar to that observed in the case of polynucleotides iii) the difference between the midpoint temperatures of the two segments of the tridecanucleotide (d(ACATGT), d(CGm^{5}CGCG)) is only about 8°C whereas the midpoint temperature of the d(CGm^{5}CGCG) hexamer is 32°C greater than that of the d(ACATGT) hexamer.

However, below 45°C the observed additional resonances demonstrate via 2D-NOESY experiment the occurence of another structure in slow equilibrium with the B form. Figure 5 shows that the additional d(m^{5}C) CH_3 resonance is insensitive to the temperature. The value of its chemical shift (1.1 ppm) is exactly the same as that observed for the same protons in the Z duplex of

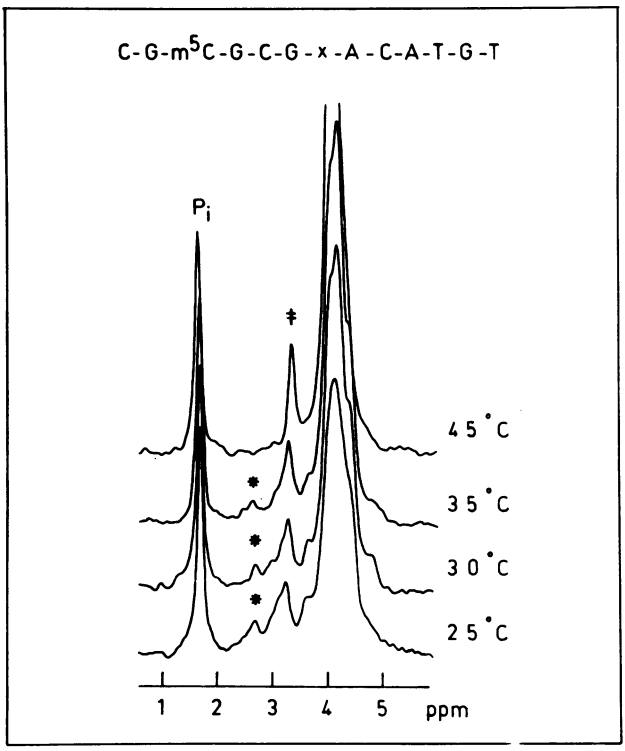


Fig. 6 Semi-log plot of the Z-B equilibrium constant (=(B)/(Z)) versus reciprocal absolute temperature.

d(CGm^{5}CGCG) in high salt concentration [23]. This value (0.5-0.6 ppm lower than in the B form) is one of the most significant features of Z protons spectra and has also been observed for the methylated pyrimidine (dm^{5}C, dT) CH_3 signals in the following Z duplexes: d(m^{5}CGCGm^{5}CG) [34], d(C-2amino-ACGTG), d(m^{5}CGCAm^{5}CGTGCG) [35], d(CGCA-m^{5}CGTGm^{5}CG) [31]. This shift has been explained theoretically by Giessner-Prettre [36]. Since in the 13-mer, the two thymidines of the d(ACATGT) part do not exhibit exchanging CH_3 signals and considering that the elementary unit of Z helices is a pyrimidine-purine dinucleotide, the present results show that the d(CGm^{5}CGCG) part of the 13-mer is involved in a B $\leftrightarrow$ Z like equilibrium whereas the d(ACATGT) part only adopts the B conformation. From integration of the CH_3 signals, the (B)/(Z) ratio (where (B) and (Z) are the B and Z duplex proportions of d(CGm^{5}CGCG) respectively) can be determined between 45° and 25°C. The Z like form represents 30% of the d(CGm^{5}CGCG) duplexes at room

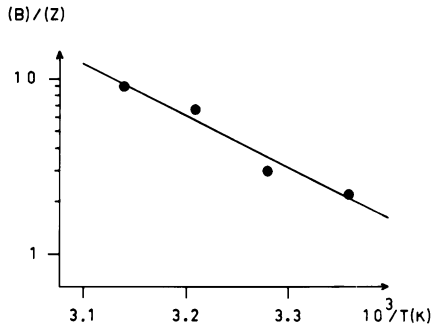


Fig. 7 36.5 MHz proton noise decoupled ^{31}P-NMR spectra of d(CGm^{5}CGCGx-ACATGT) in aqueous solution (2 mM, 0.1 M NaCl + 5 mM PO_4^{2-}) recorded at different temperatures between 45 and 25°C. The pdX (or dXp) resonance is designateed by (≠); the two Z signals of the d(CGm^{5}CGCG) part are designated by (*) (see text).

temperature. Figure 6 plots Log (B)/(Z) versus reciprocal absolute temperature. The slope of this curve gives a Z-B transition enthalpy of about 13 Kcal/mole. This value obtained in a 0.1 M NaCl solution is greater than that observed for the Z-B equilibrium of d(CGm^{5}CGCG) and d(m^{5}CGCGm^{5}CG) in 2 M NaCl solutions (7.5 and 8 Kcal/mole respectively [23,34] and for the Z-B equilibrium of d(m^{5}CGCAm^{5}CGTGCG) and d(CGCAm^{5}CGTGm^{5}CG) in 4 M NaCl solutions (about 2 Kcal/ mole) [31].

Finally, the formation of an ordered concatemer explains why the d(CGm^5-CGCG) segment of the sequence can adopt a Z form at low salt concentration (0.1 M) whereas in the case of the d(CGm^{5}CGCG) hexamer the formation of a Z duplex requires a higher salt concentration (at last > 1 M): it is well known that the increase of the chain length favors the Z form.

In order to confirm these ^{1}H-NMR results, ^{31}P-NMR spectra of d(CG-m^{5}CGCGxACATGT) were recorded at different temperatures.

^{31}P-NMR studies

Figure 7 shows the proton noise decoupled ^{31}P-NMR spectra of d(CGm^5-CGCGxACATGT) recorded between 45° and 25°C. At 45°C, two separate peaks are observed: a major resonance at 4.2 ppm and a smaller one located at higher field (3.2 ppm). Integration of these signals indicates that the latter peak corresponds to one of the expected twelve resonances. Owing to the presence of an apurinic residue (x) this peak is assigned to either px or xp phosphate group. As in the ^{1}H-NMR spectra, below 45°C a new resonance appears and is located at higher field far from the major peak ($\Delta\delta$ = 1.25 ppm). The intensity

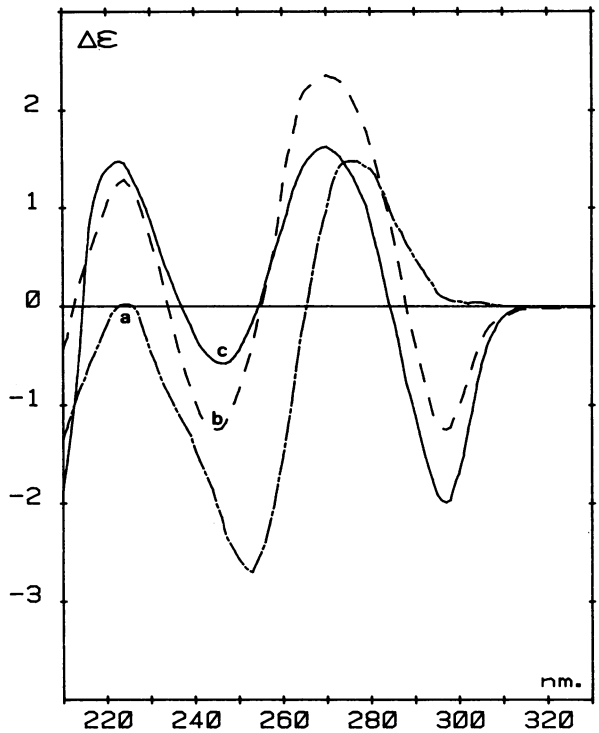


Fig. 8 CD spectra of d(CGm^{5}CGCGxACATGT) recorded at 10°C in 5 mM tris buffer, pH 7.6
a) .... 0.1 NaCl
b) – – 4 M NaCl
c) —— 4 M NaCl, 40 mM $NiCl_2$

of this additional resonance increases on lowering the temperature. At 25°C, the intensity ratio between this peak and the other signals is about 0.07.

It is well-known that in the case of a B duplex, all the ^{31}P resonances are located in a narrow region (0.7 ppm) whereas in the case of a duplex, two distinct sets of resonances are observed: one coincides approximatively with the B signals, the other is located at higher field (about 1.5 ppm far from the B resonances) [37,38]. In the ^{31}P-NMR spectrum of d(m^{5}CG)$_3$ Z duplex [38] two resonances among the expected five signals are located in the high field region. In the present study of the 13-mer, ^{1}H-NMR results showed that the Z like form represents about 30% of the d(CGm^{5}CGCG) duplexes at room temperature. According to the above remarks, at room temperature, the ^{31}P-NMR 13-mer spectrum should display two resonan-

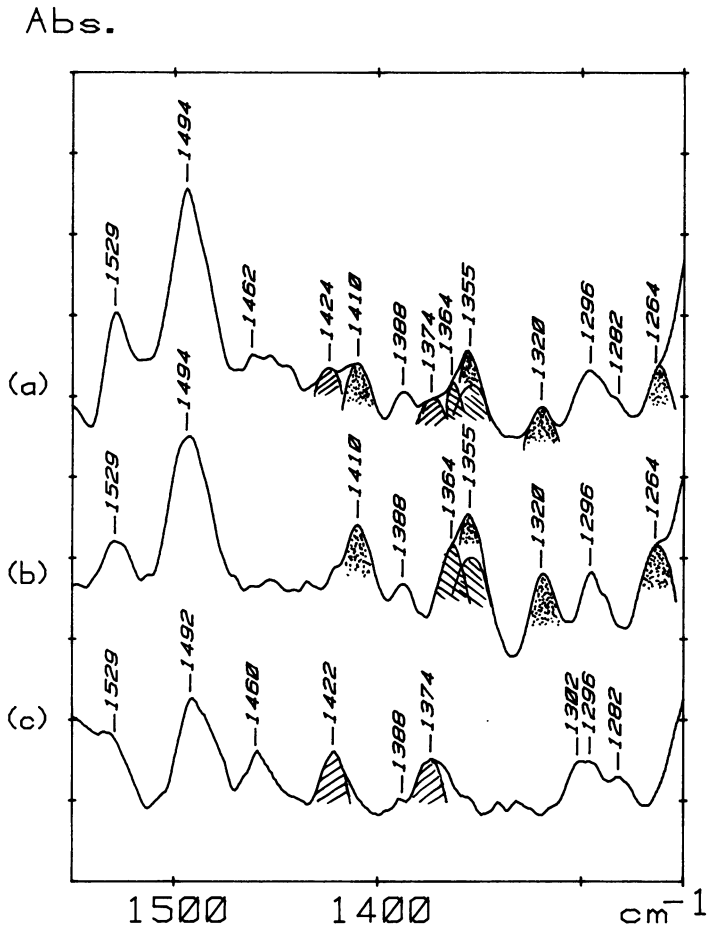


Fig. 9 Infrared spectra in the spectral range 1550 to 1250 cm^{-1}
a) tridecamer (RH 93%) coexistence of the B and Z forms
b) d(CGm^{5}CGCG) Z form (RH 66%)
c) d(ACATGT) B form (RH) 93%)

ces separated by 1.5 ppm, the intensity ratio between these two signals should be: (2 x 0.3)/(12 - 2 x 0.3) = 0.05. The experimental spectrum is in agreement with this prediction.

Circular dichroism results

In dilute solution, we have recorded at 0.1 and 4 M NaCl, the CD spectra of d(CGm^{5}CGCGxACATGT). Figure 8a shows that in 0.1 M NaCl, the classical CD spectrum of the B form is predominant. However when the NaCl concentration is increased to 4 M NaCl, a negative band is observed at 295 nm while the

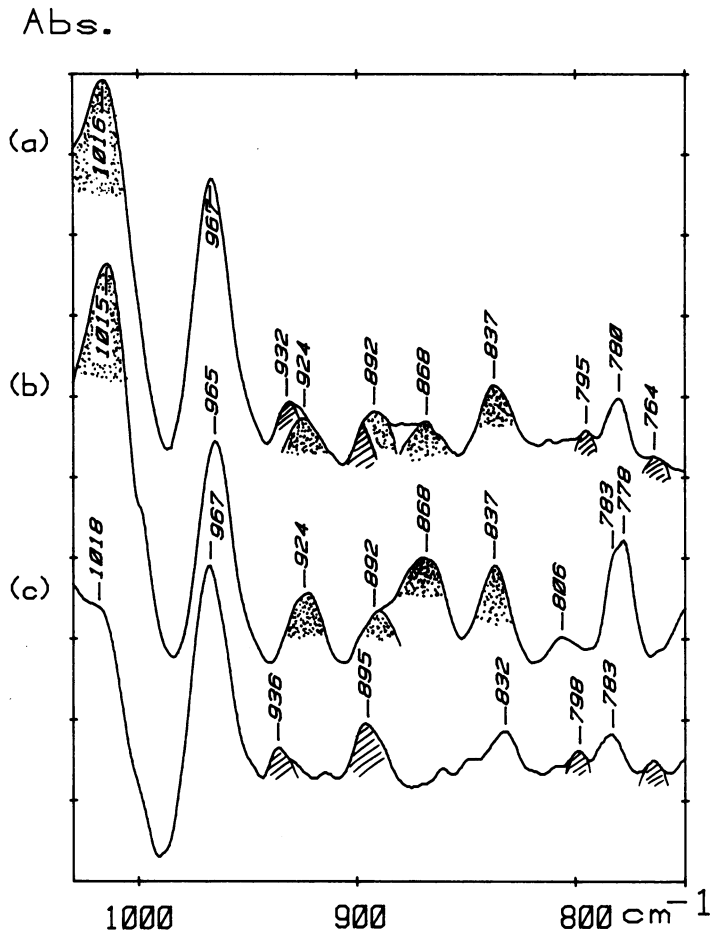


Fig. 10 Infrared spectra in the spectral range 1025 to 750 cm^{-1}
a) tridecamer (RH 93%)
b) d(CGm^{5}CGCG) Z form (RH 66%)
c) d(ACATGT) B form (RH 93%)

short wavelength CD band is depressed (Fig. 8b). B-DNA segments and Z-DNA segments are respectively attributed to the d(ACATGT) and d(CGm^{5}CGCG) sequences. This interpretation is supported by comparison with the CD spectra obtained in the same conditions of dilution and salt content, separately for each of both hexamers. At low DNA concentration, the d(ACATGT) sequence does not present any indication of a Z conformation at 4 M NaCl, while the d(CGm^{5}CGCG) sequence adopts the Z conformation [23].

A substantial change is detected after the addition of $NiCl_2$ to the 4 M

NaCl 13-mer solutions. The short wavelength CD band is more reduced and the negative 295 nm CD band is increased (Fig. 8c). Thus we can assume that the nickel ions in presence of sodium ions induce the transition of the d(ACATGT) part toward the Z conformation.

Infrared results

(1) d(CGm^{5}CGCGxACATGT) films with Na^+ counterions

Infrared spectra of d(CGm^{5}CGCGxACATGT) hydrated films with a salt content of 1 NaCl in excess per nucleotide are shown between 1550 cm^{-1} and 1250 cm^{-1} in Fig. 9a and between 1025 cm^{-1} and 750 cm^{-1} in Fig. 10a. The tridecamer spectra in the presence of Na^+ ions, whatever the relative humidity, reflect the coexistence of B and Z conformations. This can be shown by comparison with the IR spectra of the d(CGm^{5}CGCG) and d(ACATGT) hexamers recorded under identical conditions (Fig. 9b, 10b and 9c, 10c).

We have previously observed that with 1 Na^+ in excess per nucleotide, the spectra of the $d(G\text{-}C)_n$ sequence at relative humidities lower than 93% reflect a Z structure [39-41] and the spectra of the same 5-methylated sequence are always characteristic of a Z structure, whatever the relative humidity [42,43]. 5-methylation of cytosine is known to stabilize the conformation [20] and leads to observe only the Z form of $d(m^5CG)_n$ and d(CGm^{5}CGCG) films. On the contrary, an alternating purine-pyrimidine sequence containing A-T base pairs, even in a condensed phase adopts the Z form only with more difficulty. In hydrated films with Na^+ ions, the $d(A\text{-}C)_n.d(G\text{-}T)_n$ sequence has been observed in a right handed form [44]. It has been shown that in the 1550-1250 cm^{-1} spectral region, two new IR bands appear at 1320 cm^{-1} and 1264 cm^{-1} in the Z form spectrum and that the wavenumbers of two other bands depend whether the glycosidic linkage is syn or anti: the 1420 cm^{-1} band (B form) is shifted to 1410 cm^{-1} (Z form) and the 1374 cm^{-1} guanine vibration coupled to the glycosidic bond stretching (B form) to 1354 cm^{-1} (Z form). The same vibration is located at 1356 cm^{-1} for the methylated cytosine; thus the guanine and the methylated cytosine contributions overlap in the Z form spectrum at 1355 cm^{-1} [42]. An 1320 cm^{-1} band and a shoulder near 1264 cm^{-1} are detected in IR spectra of the 13-mer and of the d(CGm^{5}CGCG) films (Fig. 9a, 9b) but not in the case of d(ACATGT) (Fig. 9c). Both 1410 cm^{-1} and 1424 cm^{-1} absorption bands are found in the 13-mer spectrum (Fig. 9a). As expected, the 1410 cm^{-1} band is also present in the d(CGm^{5}CGCG) spectrum (Fig. 9b) but absent in the (ACATGT) spectrum (Fig. 9c). The shape of the absorption around 1370-1350 cm^{-1} in the 13-mer spectrum is in good

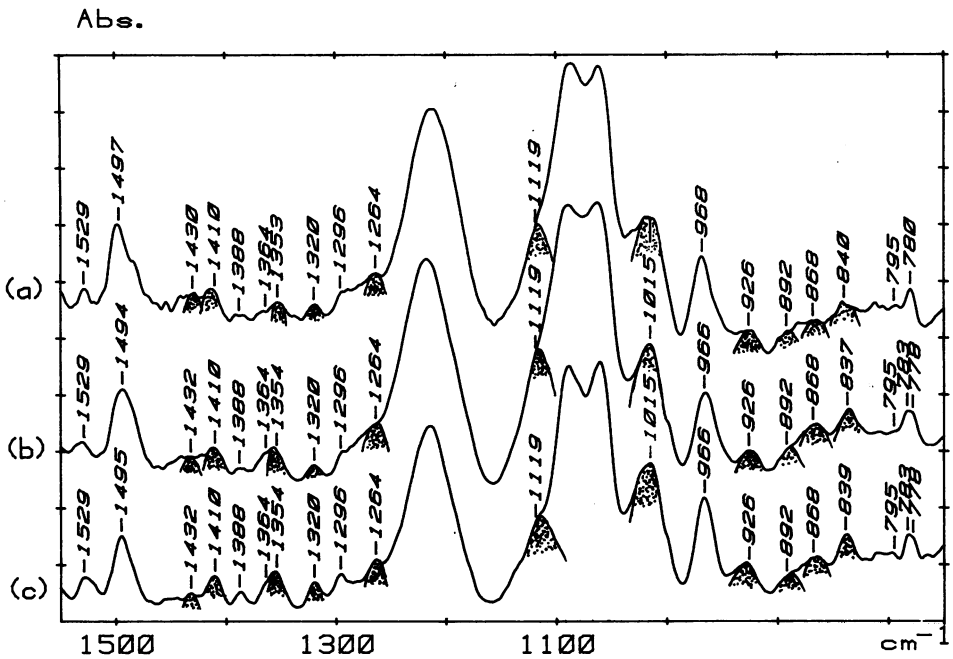


Fig. 11 Spectral region between 1550 cm^{-1} and 750 cm^{-1}. Infrared spectra of Z forms induced by Ni^{2+} ions:
a) tridecamer (RH 55%)
b) d(CGm^{5}CGCG) (RH 66%) + d(ACATGT) (RH 15%)
c) d(CGm^{5}CG) (RH 66%) + poly (dA-dC).poly (dG-dT) (RH 66%)

agreement with the recalculated spectrum obtained by addition of the Z form spectrum of d(CGm^{5}CGCG) and of the B form spectrum of d(ACATGT).

The d(CGm^{5}CGCGxACATGT) spectrum in the spectral region characteristic of the sugar phosphodiester backbone (shown in detail Fig. 10a) demonstrates also that a Z-DNA segment exists, contiguous to a B-DNA segment in the 13-mer. The Z form spectrum exhibits a medium intensity band at 924 cm^{-1} and only a very weak absorption band at 892 cm^{-1}, whereas in the B form spectrum no absorption would be detected at 924 cm^{-1} (a weak absorption exists near 936 cm^{-1}) and a more intense band would be found at 895 cm^{-1} [40,44,]. The B form has been characterized by one band near 830 cm^{-1} [44,45] and the Z form by the simultaneous presence of the 868 cm^{-1} and 837 cm^{-1} bands, related to the alterning geometry of the backbone. The 924 cm^{-1} band of the 13-mer spectrum seems well correlated with the absorption found at the same wavenumber in the d(CGm^{5}CGCG) spectrum (Fig. 10b). The main bands of the tridecamer spectrum are observed at the same wavenumbers as for the

computed spectrum using the 895 cm^{-1} and 832 cm^{-1} bands of the B form spectrum of the d(ACATGT) sequence (Fig. 10c) and the 868 cm^{-1} and 837 cm^{-1} bands of the Z form spectrum of the d(CGm^{5}CGCG) sequence (Fig. 10b). It has been impossible to obtain an A form spectrum of d(ACATGT) films by lowering the relative humidity. This short sequence which contains 66% of A-T base pairs does not undergo the B→A transition. Thus we have computed spectra by addition of the A or Z form spectrum of $d(A\text{-}C)_n.d(T\text{-}G)_n$ and of the Z form spectrum of the d(CGm^{5}CGCG) hexamer. These recalculated spectra do not reproduce the experimental 13-mer spectrum in presence of Na^+.

(2) d(CGm^{5}CGCGxACATGT) films with Ni^{2+} ions

The Z form IR spectrum has been obtained for the $d(A\text{-}C)_n.d(G\text{-}T)_n$ films in presence of Ni^{2+} [44]. As divalent transition metal ions such as Ni^{2+} would be able to stabilize the Z conformation, we have recorded the spectrum of the 13-mer with Ni^{2+} as counterions. This spectrum (Fig. 11a) is very similar to the recalculated and scaled spectrum obtained by addition of Z spectra of d(CGm^{5}CGCG) Na^+ and of d(ACATGT) Ni^{2+} (Fig. 11b). Both spectra exhibit absorption bands at 1410 cm^{-1}, 1354 cm^{-1}, 1320 cm^{-1}, 1264 cm^{-1} (shoulder), 1015 cm^{-1}, 926 cm^{-1} and they have no band at 895 cm^{-1}. The simulated spectrum shown Fig. 11c, obtained by addition of the d(CGm^{5}CG) Na^+ Z form spectrum and of the $d(A\text{-}C)_n.d(G\text{-}T)_n$ Ni^{2+} also reproduces the spectrum of the 13-mer. Concerning the absorptions of the A-T base pairs, the effect of the right-handed → left-handed helix transition is detected in the Z form spectrum of $d(A\text{-}C)_n.d(GT)_n$ Ni^{2+} film, thanks to a band located at 1434 cm^{-1}. This band exists in the d(CGm^{5}CGCGxACATGT) Ni^{2+} spectrum as in the two recalculated spectra in Fig. 11 b and 11c. Thus we can assume that the nickel ions have induced the Z conformation in the d(ACATGT) part of the tridecamer.

CONCLUSION

The CD, NMR and IR results justify the choice of the 13-mer sequence and confirm our predictions: a right-handed and a left-handed conformation can coexist within the same DNA molecule, via a single apurinic residue. However, the Z proportion of the d(CGm^{5}CGCG) segment depends on the experimental conditions. The Z form is only detected by CD techniques at high salt concentration whereas about 30% of Z duplex is observed by NMR at room temperature in 0.1 M NaCl solution. In films, the d(CGm^{5}CGCG) segment is always in a Z form at low humidity. As pointed out in a previous paper [35,40] the discrepancy between the CD, NMR and IR data are explained by the various

oligomer concentration used in these techniques since the increase of DNA concentration favors the Z form. For instance, the concentration used in CD experiments is one to two orders of magnitude lower than that used in NMR experiments.

Examination of the base pairing process of the 13-mer shows that each of the two duplex units (d(ACATGT) and d(CGm^{5}CGCG)) are surrounded by a missing residue on one side and by a no-base residue on the other. Thus our results show that such alterations of biological importance involving one base pair lead to a flexible junction between B ans Z forms in an appropriate base sequence. More investigations with other synthetic oligonucleotides are undertaken in order to confirm a model of B-Z junction with an apurinic site.

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