A → Z transition in the synthetic hexanucleotide $(dCdGfl)_3$

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500 MHz proton NMR and NOE measurements on $(dCdGfl)_3$ show that at very low ionic strength the hexanucleotide adopts an A-DNA conformation, whereas at high salt concentrations a Z-form is found. At intermediate salt concentrations the two species are in slow exchange on the proton NMR time scale. This transition was also observed by characteristic changes in the CD spectra.

NMR NOE 2'-Deoxy-2'-fluoronucleoside CD Conformation

1. INTRODUCTION

It is now well established that the sugar conformation determines the structure and conformation of oligo- and polynucleotides [1]. The sugar conformation is in turn determined by the size and particularly by the polarity of the 2'-substituent [2]. It has been shown [3,4] that the more polar the substituent, the more the conformational equilibrium is displaced towards the 3'-*endo* (N) form. Thus, 2'-deoxy-2'-fluoronucleosides all show a large preference for the 3'-*endo* ribose type conformer, despite the small size of the fluorine atom. This property is strongly preserved and even increased in polynucleotides, the duplexes of which are considerably more stable than their ribo- or deoxyribo counterparts [5–7].

Two major conformational differences distinguish the left-handed Z-DNA from the right-handed forms A and B. While in the latter all bases are in the *anti* orientation and the sugar conformations are the same in a given form, i.e., 2'-*endo* in B-DNA and 3'-*endo* in A-DNA, Z-DNA shows a characteristic alternation of both glycosidic torsion angle and sugar conformation: the deoxycytidines show *anti* orientation and 2'-*endo* puckering and the deoxyguanosines are in *syn* orientation with 3'-*endo* pucker [8]. This peculiar alternating arrangement is responsible for the zig-zag structure of Z-DNA.

We therefore decided to study a hexanucleotide where the transition to the Z-form may be facilitated. Since the free nucleoside dGfl has been found to be preferentially in the 3'-*endo* conformation [9] in solution and in a multitude of conformations in the crystal state [10], we chose to synthesize and study $(dCdGfl)_3$.

Abbreviations: dGfl, 2'-deoxy-2'-fluoroguanosine; NOE, nuclear Overhauser effect; CD, circular dichroism

2. MATERIALS AND METHODS

dGfl was synthesized as described in [9]. The oligonucleotide was constructed by a modified triester method [11].

NMR spectra were recorded on a Bruker WM500 spectrometer in D_2O buffers as indicated. The NOEs were observed by cycling 16 transients on resonance followed by 16 transients off resonance.

CD spectra were recorded in 2-mm cuvettes in a thermostatted cell holder on a Jobin-Yvon Mark V dichrograph.

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3. RESULTS

3.1. *Low salt form*

The resonances of the G(H^8) and C(H^6) protons are observed in the range 7.4–8.0 ppm. The C(H^6) protons are readily distinguished by their doublet structure from coupling to C(H^5), $^3J \sim 7$ Hz (fig.1). In the range 4.9–6.2 ppm, the 6 anomeric proton resonances, the 3 C(H^5) resonances and the 3 G(H_2') resonances are observed. The C(H^5) resonances can readily be distinguished from their coupling to C(H^6), the G(H_1') resonances show vicinal coupling to ^{19}F of 15–22 Hz and geminal ^{19}F coupling of ~52 Hz is observed to G(H_2') [4,9]. The 3 remaining multiplets correspond to the cytidine anomeric protons. The minor resonances observed in fig.1 arise from the species which is dominant at high salt concentration (see below).

Preirradiation of the G(H^8) or C(H^6) resonances gives rise to small (2.5–6%) NOEs on the anomeric protons, much smaller than the NOEs observed between C(H^6) and C(H^5) protons (~20%). These results are consistent with an *anti*, but not with a *syn* conformation about the glycosidic bond for all the residues.

In a right-handed helix (A- or B-form) intraresidue NOEs to the 1′, 2′ and 2′′ proton resonances and interresidue NOEs to the same protons of the residue in the 5′-direction will be observed upon irradiation of the base H^8 or H^6 resonances [12]. Irradiation of all 3 G(H^8) resonances and of two of the C(H^6) resonances (7.50–7.52 ppm) gives rise to NOEs to two anomeric protons and to the 2′ protons of both a cytidine and a guanosine residue. Irradiation of the C(H^6) resonance at 7.96 ppm gives NOEs only to its own anomeric proton and 2′ and 2′′ resonances, which identifies it as the terminal residue and shows that the helix is right-handed. For a left-handed Z-form helix the 3′-terminal (^{6}G) residue would give rise to no interresidue sugar proton NOE.

For a right-handed helix the 2′ proton is closer to H^8 or H^6 than the 2′′ proton in the same residue, both for an A- or a B-form [12]. Thus, irradiation of the C(H^6) proton resonances for short ($\leq$150 ms) irradiation times readily distinguishes the 2′ and 2′′ protons. However, the interresidue NOEs expected between G(H^8) and the cytidine 2′ and 2′′ protons of the residue in the 5′-direction are quite different for an A- or B-form helix [12]. For the B-form the cytidine 2′′ proton is much closer to G(H^8) than the 2′ proton, whereas for an A-form the order is reversed.

Fig.2 shows the observed NOEs from irradiation of ^{3}C(H^6) and ^{4}G(H^8) resonances. From irradiation of ^{3}C(H^6), the resonance at 2.69 ppm can be assigned to the 2′ proton as it gives the largest NOE. A larger NOE is also observed on the resonance at 2.69 ppm (H_2') than at 2.58 ppm (H_2'') upon irradiation of ^{4}G(H^8).

The same relative NOE effect is observed for each dCpdGfl unit and shows that the helix adopts an A-form.

3.2. *High salt form*

On raising the salt concentration the minor resonances observed in fig.1 increase in intensity, while those attributed to the A-form decrease. At the oligonucleotide concentration of the NMR

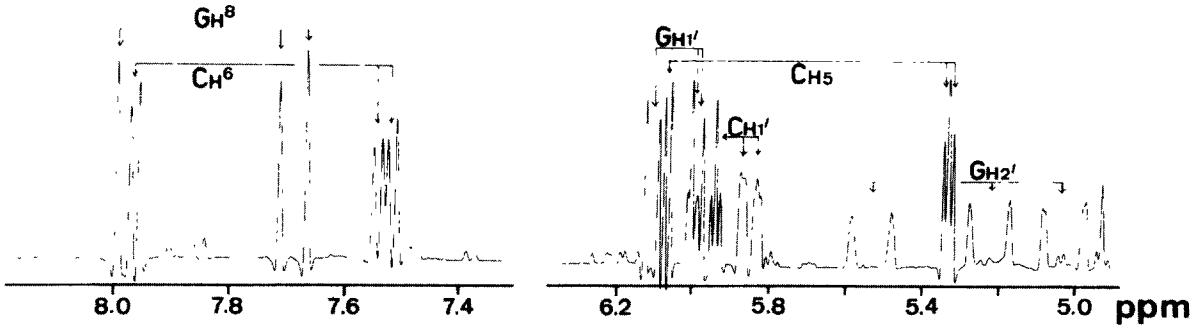

Fig.1. Resolution enhanced 500 MHz NMR spectrum of $(dCdGfl)_3$ in 10 mM phosphate, pH 7.2, at 15°C. Only resonances to low field of the residual water peak are shown. The minor resonances correspond to the high salt form.

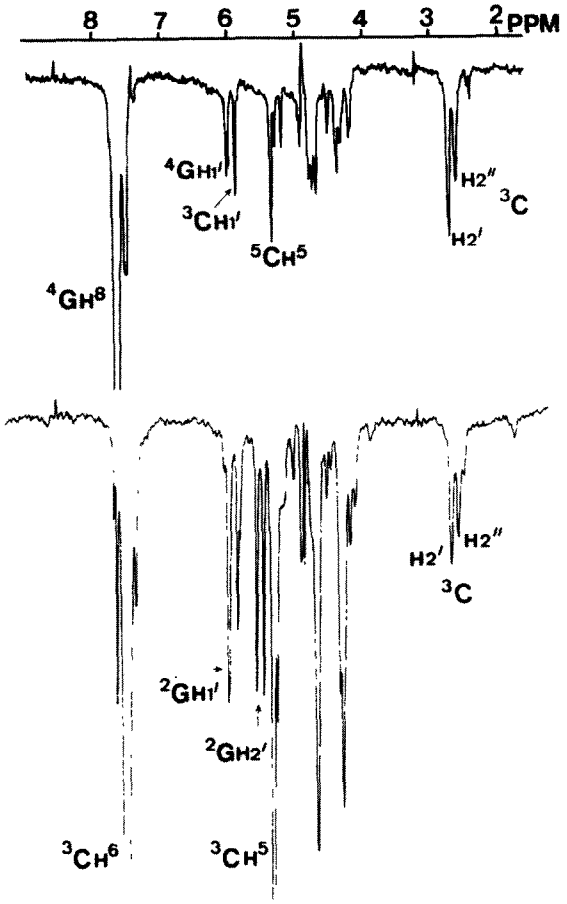

Fig.2. NOEs observed on preirradiation of $^{3}C(H^{6})$ (lower) and $^{4}G(H^{8})$ (upper) of $(dCdGfl)_3$ for 0.5 s at 15°C in 10 mM phosphate buffer.

samples the transition is not very cooperative and the A-form only disappears at 1.5 M NaCl at 20°C. At intermediate salt concentrations exchange between the two different forms of the hexanucleotide is slow on the proton NMR time scale.

The low-field part of the spectrum is shown in fig.3. In the aromatic region the 3 $G(H^{8})$ resonances are resolved while the 3 $C(H^{6})$ resonances are unfortunately coincident. Upon irradiation of each of the $G(H^{8})$ resonances a very large NOE (~30%) to a guanosine H_1' is observed, while no NOE to the cytidine H_2' and H_2'' is found. The magnitude of the NOE on the guanosine anomeric proton resonances is almost as

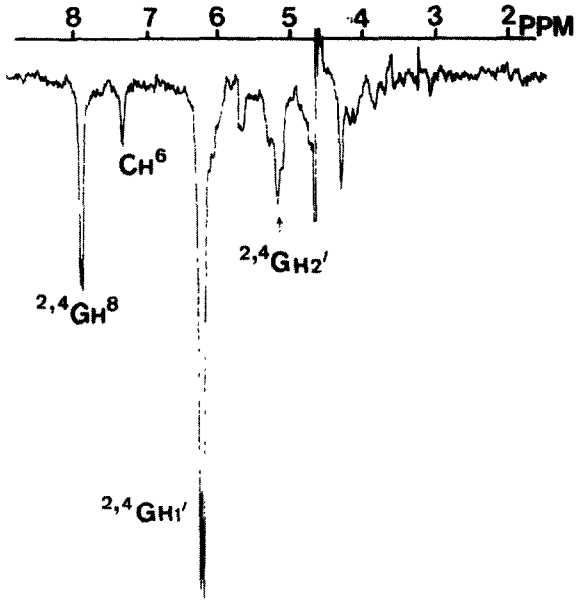

Fig.4. NOEs observed on preirradiation of the two internal guanosine anomeric protons of $(dCdGfl)_3$ for 0.3 s at 20°C. Solution 10 mM phosphate, 2 M NaCl, pH 7.2.

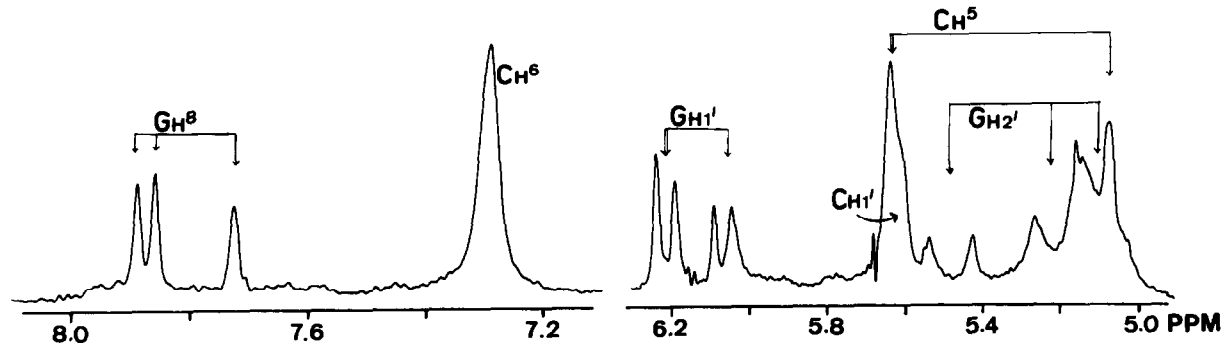

Fig.3. Slightly resolution enhanced NMR spectrum of $(dCdGfl)_3$ in 10 mM phosphate, 2 M NaCl at 20°C.

large as that between C(H^5) and C(H^6) resonances and shows that guanosine assumes the *syn* conformation.

Irradiation of the two internal guanosine anomeric protons (fig.4) gives large NOEs to the corresponding G(H^8) resonances (34%) and to the G(H_2') resonances (37%), indicating that these interproton distances must be very similar. An NOE is observed on the C(H^6) resonance (7%). This is consistent with the fairly short (3.7 Å) distance observed to the C(H^6) of the cytidine residue in the 3'-direction found in the Z-form of d(C-G)$_3$ [13].

3.3. *CD spectra*

The results of the CD spectra agree with those obtained by NMR. At very low salt concentrations (1 mM NaCl) and low temperature the CD spectrum of (dCdGfl)$_3$ shows a broad positive band around 275 nm and a much smaller negative one around 290 nm. Both bands increase in intensity upon raising the salt concentration to 0.1 M NaCl. This CD spectrum is reminiscent of that of the A-form of r(C-G)$_3$ [14,15]. This spectrum becomes even more pronounced at low temperature (fig.5a), with several characteristic bands (large positive band at 270 nm, negative bands at 210, 230, 250 and 290 nm), virtually the same as that of r(C-G)$_3$ [14,15], demonstrated to be that of the A-form. Increasing the ionic strength above 1.5 M NaCl changes the CD spectrum further: the large positive band decreases and shifts towards lower wavelengths, while the negative bands below 250 nm disappear in favor of a small positive band around 225 nm (fig.5b), very similar to the Z-form spectrum of d(C-G)$_2$ and d(C-G)$_3$ [16,17]. Interestingly enough, at very high ionic strength, the A-form does not appear during melting. The equilibrium between A- and Z-forms appears to exist only between 0.1 and 1.5 M NaCl.

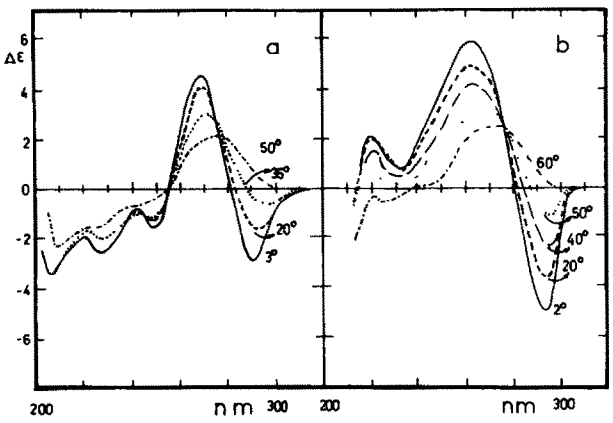

Fig.5. (a) CD spectra of (dCdGfl)$_3$ in 0.1 M NaCl, 10 mM cacodylate, pH 7.5 at temperatures indicated. (b) CD spectra of (dCdGfl)$_3$ in 4.5 M NaCl, 10 mM cacodylate at temperatures indicated.

4. DISCUSSION

We have chosen to study the mixed hexamer (dCdGfl)$_3$ to determine whether the predominant conformation of the free nucleosides, i.e., 2'-*endo* in dC and 3'-*endo* in dGfl which are those found in Z-DNA, will favor the formation of this form. The present data appear to confirm this.

(dCdGfl)$_3$ is, however, an example of an A⟶Z transition. The thermodynamic preference of dGfl for the 3'-*endo* conformation [3,4] apparently imposes the A-form on the hexanucleotide and favors the induction of the Z-form under the influence of increased salt concentration. The absence of the B-form in (dCdGfl)$_3$ requires explanation. Despite the kinetic flexibility of the 2'-deoxy-2'fluoronucleosides [10,18] and the well known adaptability of deoxyribonucleosides, the A-form appears to dominate.

The transition between the A- and Z-form of (dCdGfl)$_3$ is, however, not very cooperative. Even in 0.15 M NaCl at low temperature a small amount of Z-DNA is present (fig.1). It is only above 1 M NaCl that the Z-form is predominant (figs 3, 4, 5b) and it is complete above about 1.5 M salt. At all ionic strengths the exchange between A- and Z-forms is slow on the NMR time scale, indicative of a rather large activation energy accompanying the right-to-left transitions.

Our results indicate the importance of the sugar conformation in structural transitions even in small oligonucleotides.

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