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B and Z double helical conformations of d-(m⁵C-G-C-G-m⁵C-G) in aqueous solution

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B,Z-DNA conformation *CD* *¹H-NMR*

1. INTRODUCTION

The conformation transition between R and L (two different double helical forms) of poly d(GC) has been observed as a function of sodium salt concentration for the first time in 1972 [1] using ORD, CD and UV techniques. The L form was later attributed to the Z form observed in a X-ray diffraction analysis of d(CG)₃ [2,3] and d(CG)₂ [4,5]. It has been shown [6] that the methylation of the cytosine on the 5 position in poly d(G-m⁵C) favoured the Z conformation which was formed at a much lower salt concentration than was the case for unmethylated polymer poly d(GC) [7]. Recently authors in [8] observed the Z form with a methylated hexamer d(m⁵CG)₃ in the solid state. However, the high stabilisation of the Z form in aqueous solution due to the methyl group at the 5 position on cytosine has not yet been proved for short deoxy-oligomers in solution. On the other hand, the B-Z transition of d(CG)₃ takes place not only at high sodium salt concentration but also at low temperatures [9]; this makes it difficult to

observe the ¹H-NMR signals, especially when only a small fraction of the compound adopts the Z form.

In order to obtain a high proportion of the Z form at room temperature and facilitate the ¹H-NMR assignment, the hexamer d(m⁵C-G-C-G-m⁵C-G) methylated at the 5 position of two external dC residues was synthesized and the B and Z forms of this deoxyoligomer subsequently studied in aqueous solution at various salt concentrations and temperatures by CD and ¹H-NMR techniques.

2. MATERIALS AND METHODS

d(m⁵C-G-C-G-m⁵C-G) was prepared in solution from methyl-5 deoxycytidine [10] with triisopropylbenzenesulfonyl nitro-triazole as the coupling agent. It was deprotected and purified as in [11]. Circular dichroism was measured with an Autodichrograph Mark V (Jobin Yvon) spectropolarimeter. Samples for CD spectroscopy were prepared by diluting the oligomer in a phosphate buffer 3 M NaCl. The CD spectra were recorded after heating the solution to 60°C for 10 min and then cooling it to room temperature for measurement. The ¹H-NMR experiments were performed at 500 MHz in the Fourier transform mode with a Bruker WM 500 spectrometer. The hexamer

Abbreviation: d-(m⁵C-G-C-G-m⁵C-G), 2'-deoxy 5-methyl cytidyl (3'-5') deoxyguanylyl (3'-5') deoxycytidyl (3'-5') deoxyguanylyl (3'-5') deoxy 5-methyl cytidyl (3'-5') deoxyguanine

was dissolved in $^2\text{H}_2\text{O}$ containing 0.1 M (or 2 M) NaCl + 5 mM PO_4^{2-} and was freed of possible divalent ions by shaking with Chelex 100 followed by the addition of EDTA (≈ 0.1 mM). The pH was adjusted to 7–8 by adding a small amount of NaOH. The samples were lyophilized twice in $^2\text{H}_2\text{O}$ and redissolved in $^2\text{H}_2\text{O}$ at a final concentration of 1–2 mM. These solutions were introduced into NMR tubes which were then degassed in a vacuum line and sealed. The chemical shifts were measured from 3-(trimethylsilyl)[$^2\text{H}_4$]propionic acid.

3. RESULTS AND DISCUSSION

3.1. Circular dichroism studies

The circular dichroism spectrum of $\text{d}(\text{m}^5\text{C-G-C-G-m}^5\text{C-G})$, dissolved in H_2O containing 0.1 M NaCl + 5 mM PO_4^{2-} at pH 7.5 and room temperature is shown in fig. 1a. This CD spectrum, characterized by an important negative maximum at 257 nm and a positive maximum at about 282 nm, is similar to that of poly d(G-C) [1] and

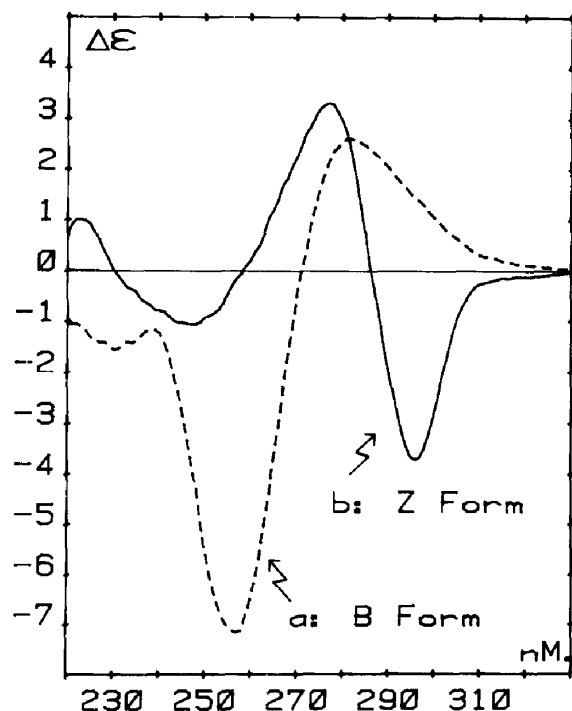


Fig.1. Circular dichroism spectra of $\text{d}(\text{m}^5\text{C-G-C-G-m}^5\text{C-G})$ in H_2O containing: (a) 0.1 M NaCl; (b) 3 M NaCl at room temperature.

poly d(G-m 5 C) [6] in the B form at low salt concentration. The CD spectrum of $\text{d}(\text{m}^5\text{C-G-C-G-m}^5\text{C-G})$ in 3 M NaCl is quite different (fig. 1b). It presents a positive peak at 277 nm and a negative peak at 296 nm indicating that the Z form is stable and presents at least 80% proportion at room temperature. In the 2 M NaCl solution, the CD spectrum (now shown) reveals the presence of the B and Z form in equilibrium below $t < 50^\circ\text{C}$ whereas the negative peak at 296 nm, characteristic of the Z form, disappears at higher temperature ($t > 60^\circ\text{C}$). In contrast, for the unmethylated hexamer, $\text{d}(\text{C-G})_3$, the Z-form was only observed in 4 M NaCl in a much lower proportion (about 25%) at room temperature [9]. Thus the methylation of the cytosine in short alternating dC.dG fragments clearly favours the stabilisation of the Z form in solution in agreement with the result obtained for the methylated polymer, $\text{d}(\text{G-m}^5\text{C})$. It seems likely that the methylation of the cytosine shifts the negative peak, characteristic of the Z form to longer wave lengths: this peak is situated at about 290 nm for poly d(G-C) [1], 292 nm for poly d(G-m 5 C) [6] and 296 nm for $\text{d}(\text{m}^5\text{C-G-C-G-m}^5\text{C-G})$.

3.2. $^1\text{H-NMR}$ studies

Fig. 2 shows the 500 MHz $^1\text{H-NMR}$ spectra of the $\text{d}(\text{m}^5\text{C-G-C-G-m}^5\text{C-G})$ base protons obtained with a 0.1 M NaCl solution at 70 and 27°C . The assignment was performed by comparison with $\text{d}(\text{m}^5\text{C-G})$ and $\text{d}(\text{C-G-m}^5\text{C-G})$ spectra at $t > 70^\circ\text{C}$. The proton chemical shift variations at various temperatures were found to be very similar to the case of other hexamers [11–14]. At high temperature, $t > 80^\circ\text{C}$, the coil form is predominant while at room temperature the double helical form is practically 100% present. In the 20–90°C temperature interval, only one resonance signal was observed for each proton, showing a rapid exchange between the helix and coil forms; and the observed chemical shift of each of the protons therefore reflects the relative variation of the helix and coil proportions. The sum of the coupling constants involving the H_1 proton, $J_{1,2'} + J_{1,2''}$ is about 14–15 Hz and the $J_{1,2'}$ -value is about 8–9 Hz for all residues, indicating that the B form is highly predominant in the 0.1 M NaCl solution [11–13]. This conclusion is in agreement with that obtained from the above CD study (fig. 1a).

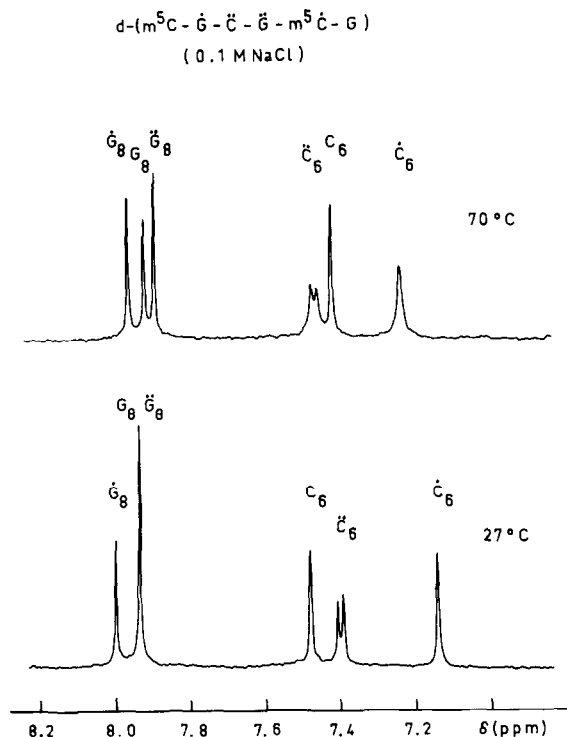


Fig.2. 500 MHz 1H -NMR spectra of $d-(m^5C-G-C-G-m^5C-G)$ base protons in a 0.1 M NaCl solution at 70 and 27°C.

In the presence of 2 M NaCl and for $t > 60^\circ C$ each proton gives rise to a single resonance signal, the chemical shift of which was found to be similar to that obtained with the 0.1 M NaCl solution. In contrast, at room temperature, two resonance signals were observed for each proton. Fig. 3a and b show the 500 MHz 1H -NMR spectra of the H_8 protons of 3 dG and H_6 protons of 3 dC residues. Fig. 4a and b show the resonances of the methyl groups at the 5 position of the two m^5dC residues at 65 and 27°C. These figures clearly indicate that at 27°C, two double helical forms are present in solution and that the exchange between these two forms is slow (> 10 s). The resonance signals corresponding to the B form were easily identified by comparison with the proton spectra obtained at 0.1 M NaCl. The additional signals were then attributed to the Z form on the basis of the above CD results. The H_6 and CH_3 resonance assignment for the two methylated cytosine residues in the Z form is still unknown at this time since the Z form of $d(m^5C-G)$ and $d(C-G-m^5C-G)$ was not detected

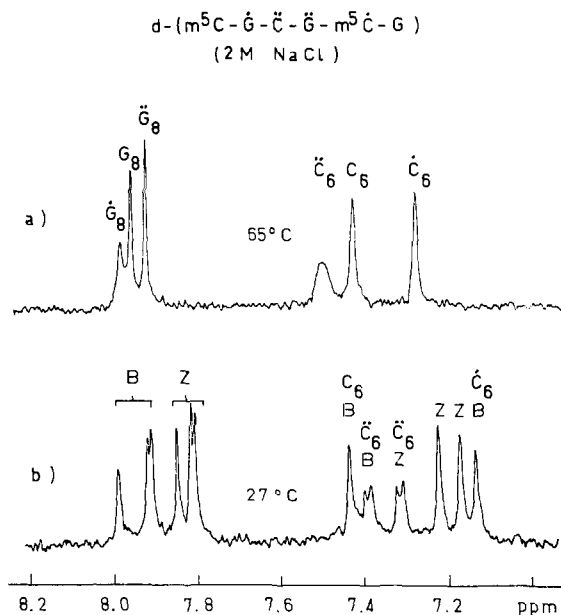


Fig.3. 500 MHz 1H -NMR spectra of $d-(m^5C-G-C-G-m^5C-G)$ base protons in a 2 M NaCl solution: (a) at $t = 65^\circ C$ and (b) at $t = 27^\circ C$.

table under the same experimental conditions.

It follows from fig.3b that for a given double helical form (B or Z) the 3 resonance signals of the $(dG)H_8$ protons are very close to one another, whereas the chemical shift of the 3 $(dC)H_6$ protons are similar to one another for the Z form and very different for the B form. In contrast, the results in fig.4b indicate that the magnetic environment of the two methyl groups should be very different for the Z form of $d(m^5C-G-C-G-m^5C-G)$. On the other hand, although the orientation of guanine with respect to the sugar ring is quite different in the B and Z forms (*anti* for the B and *syn* for the Z form), the average chemical shift difference for the $(dG)H_8$ protons in these two double helical forms is surprisingly moderate, smaller than 0.2 ppm (fig.3b), similar to the case of the $(dC)H_6$ protons. On the contrary, the situation is quite different for the methyl group of the internal m^5dC residues: the chemical shift difference between the B and Z forms is very large, about 0.55 ppm, and the methyl signal corresponding to the Z form ($\delta = 1.16$ ppm) is located at higher field far from the B form signal ($\delta = 1.71$ ppm). This suggests that the methyl group of the internal m^5dC residue is very close and situated 'inside' the guanine ring

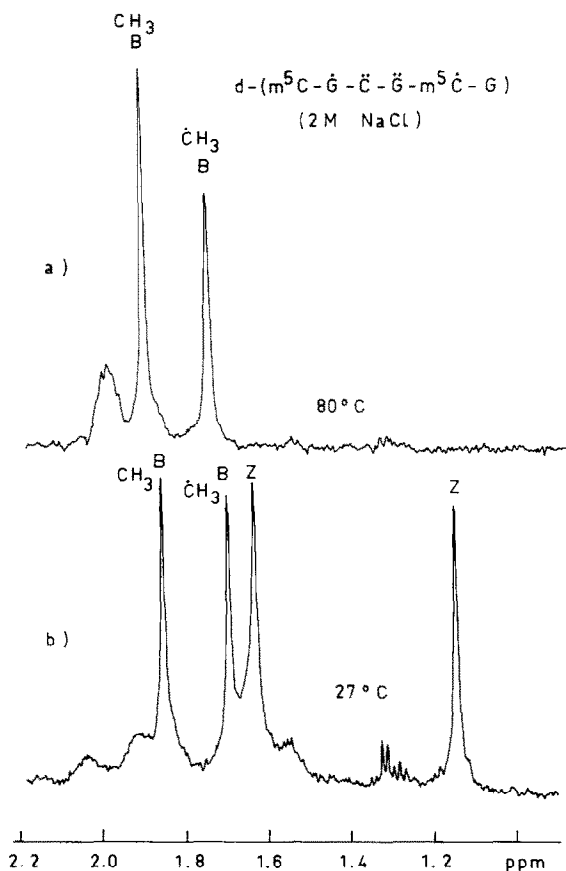


Fig.4. 500 MHz ^1H -NMR spectra of $\text{d}-(\text{m}^5\text{C-G-C-G-m}^5\text{C-G})$ of the methyl protons: (a) at $t = 65^\circ\text{C}$ and (b) at $t = 27^\circ\text{C}$.

(upfield shift) of the neighbouring dG residue. This hypothesis is supported by the recent X-ray study [8] in which it was shown that the methyl groups in $\text{d}(\text{m}^5\text{C-G})_3$ are tucked under and in close Van der Waal contact with the imidazole ring of guanine.

The B and Z proportions were determined by integrating the $(\text{dG}(\text{H}_8, (\text{dC or m}^5\text{dC})\text{H}_6 \text{ and } (\text{m}^5\text{dC})\text{CH}_3)$ signals. At 27°C , the Z proportion is slightly higher than that of the B form, about 53% for the Z and 47% for the B form.

In conclusion, the great advantage of the present hexamer, $\text{d}(\text{m}^5\text{C-G-C-G-m}^5\text{C-G})$, is that the double helical conformation changes easily from the B to the Z form when the sodium salt concentration varied between 0.1 M and 3 M. In a 2 M NaCl solution, the B and Z populations are practically equivalent at room temperature; this should facilitate comparative NMR studies on the specific interaction, in solution of peptides, proteins or antibodies with either of the double helical forms.

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