B and Z double helical conformations of d-(m^{5}C-G-C-G-m^{5}C-G) in aqueous solution

S. Tran-Dinh*, J. Taboury*+, J.-M. Neumann*, T. Huynh-Dinh, B. Genissel, C. Gouyette and J. Igolen

*Unité de Chimie Organique, ERA/CNRS 927, Institut Pasteur, 28 rue du Docteur-Roux, 75724 Paris Cedex 15, *Service de Biophysique, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette, and +Laboratoire de Spectroscopie Biomoléculaire, U.E.R. de Médecine, 74, rue Marcel-Cahin, 93012 Bobigny Cedex, France*

Received 24 January 1983; revision received 4 March 1983

Abstract not received

B,Z-DNA conformation CD ^{1}H-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-m^{5}C) 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(m^{5}CG)$_3$ 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 ^{1}H-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 ^{1}H-NMR assignment, the hexamer d(m^{5}C-G-C-G-m^{5}C-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 ^{1}H-NMR techniques.

Abbreviation: d-(m^5-C-G-C-G-m^{5}C-G), 2′-deoxy 5-methyl cytidyl (3′-5′) deoxyguanylyl (3′-5′) deoxycytidyl 3′-5′) deoxyguanylyl (3′-5′) deoxy 5-methyl cytidyl (3′-5′) deoxyguanine

2. MATERIALS AND METHODS

d(m^{5}C-G-C-G-m^{5}C-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 ^{1}H-NMR experiments were performed at 500 MHz in the Fourrier transform mode with a Brucker WM 500 spectrometer. The hexamer

Published by Elsevier Science Publishers
00145793/83/0000–0000/$3.00

was dissolved in ^2H_2O 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 ^2H_2O and redissolved in ^2H_2O 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)[$^2H_4$]propionic acid.

3. RESULTS AND DISCUSSION

3.1. *Circular dichroism studies*

The circular dichroism spectrum of d(m^{5}C-G-C-G-m^{5}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 poly d(G-m^{5}C) [6] in the B form at low salt concentration. The CD spectrum of d(m^{5}C-G-C-G-m^{5}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 $t<50°C$ whereas the negative peak at 296 nm, characteristic of the Z form, disappears at higher temperature ($t>60°C$). In contrast, for the unmethylated hexamer, d(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, d(G-m^{5}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 d(m^{5}C-G-C-G-m^{5}C-G).

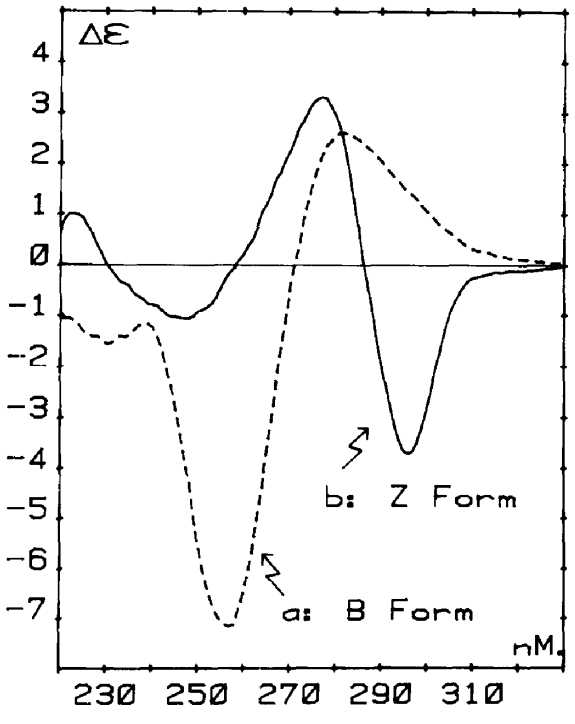

Fig.1. Circular dichroism spectra of d-(m^{5}C-G-C-G-m^{5}C-G) in H_2O containing: (a) 0.1 M NaCl; (b) 3 M NaCl at room temperature.

3.2. *^{1}H-NMR studies*

Fig. 2 shows the 500 MHz ^{1}H-NMR spectra of the d(m^{5}C-G-C-G-m^{5}C-G) base protons obtained with a 0.1 M NaCl solution at 70 and 27°C. The assignment was performed by comparison with d(m^{5}C-G) and d(C-G-m^{5}C-G) spectra at $t>70°C$. 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, $t>80°C$, 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).

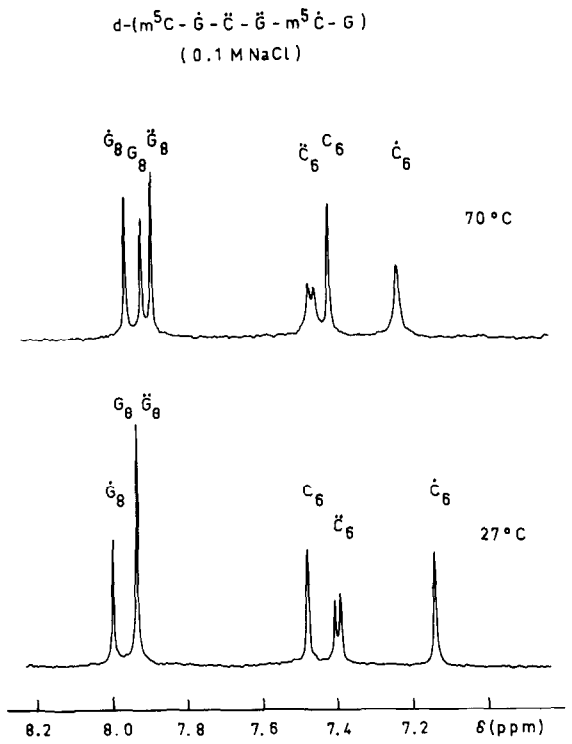

Fig.2. 500 MHz ^{1}H-NMR spectra of d-(m^{5}C-G-C-G-m^{5}C-G) base protons in a 0.1 M NaCl solution at 70 and 27°C.

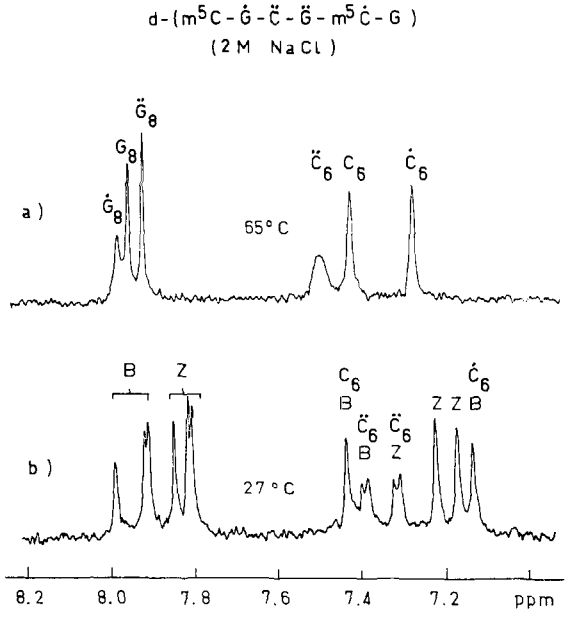

Fig.3. 500 MHz ^{1}H-NMR spectra of d-(m^{5}C-G-C-G-m^{5}C-G) base protons in a 2 M NaCl solution: (a) at $t = 65$°C and (b) at $t = 27$°C.

In the presence of 2 M NaCl and for $t > 60$°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 ^{1}H-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^{5}dC 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 basic 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^{5}C-G) and d(C-G-m^{5}C-G) was not detectable 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^{5}C-G-C-G-m^{5}C-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^{5}dC 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^{5}dC residue is very close and situated 'inside' the guanine ring

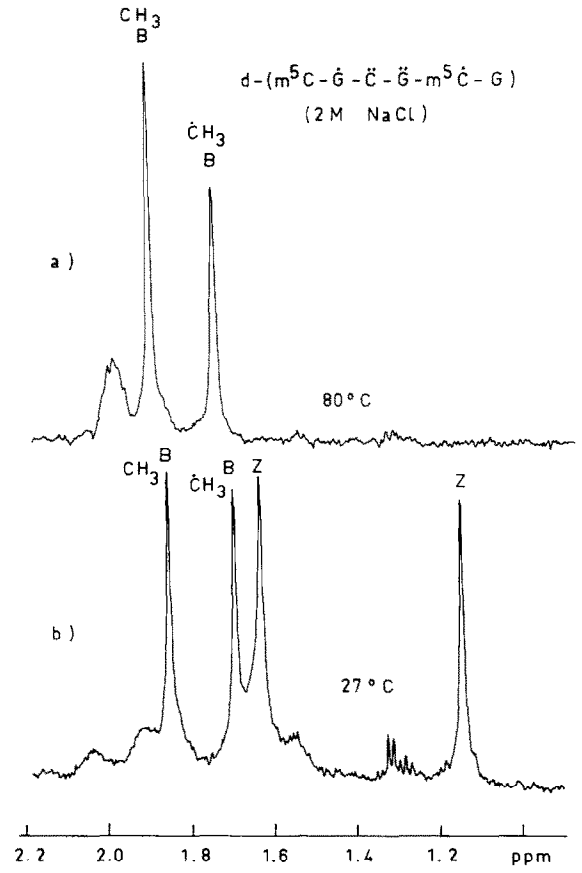

Fig.4. 500 MHz ^{1}H-NMR spectra of d-(m^{5}C-G-C-G-m^{5}C-G) of the methyl protons: (a) at $t = 65°C$ and (b) at $t = 27°C$.

(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 d(m^{5}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 (dG(H_8, (dC or m^{5}dC)H_6 and (m^{5}dC)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, d(m^{5}C-G-C-G-m^{5}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.

REFERENCES

[1] Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375-396.
[2] Wang, A.H.-J. Quigley, G.J., Kolpak, F.J., Crawford, J.L., Van Boom, J.H., Van der Marel, G.A. and Rich, A. (1979) Nature 282, 680-686.
[3] Wang, A.H.-J, Quigley, C.J., Kolpak, F.J., Van der Marel, G., Van Boom, J.H. and Rich, A. (1981) Science 211, 171-176.
[4] Drew, H., Takano, T., Tanaka, S., Itakura, K. and Dickerson, R.E. (1980) Nature 286, 567-573.
[5] Crawford, J.L., Kolpak, F.J., Wang, A.H.-J., Quigley, G.J., Van Boom, J.H., Van der Marel, G. and Rich, A. (1980) Proc. Natl. Acad. Sci. USA 77, 4016-4020.
[6] Behe, M. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sci. USA 78, 1619-1623.
[7] Behe, M., Zimmerman, S. and Felsenfeld, G. (1981) Nature 293, 233-235.
[8] Fujii, S. Wang, A.H.-J., Van der Marel, G., Van Boom, J.H. and Rich, A. (1982) Nucl. Acids. Res. 10, 7879-7892.
[9] Thamann, T.J., Lord, R.C., Wang, A.H.-J. and Rich, A. (1981) Nucleic Acids. Res. 9, 5443-5457.
[10] Sung, W.L. (1981) J. Chem. Soc. Chem. Commun. 1089.
[11] Tran-Dinh, S., Neumann, J.M., Huynh-Dinh, T., Allard, P., Lallemand, J.Y. and Igolen, J. (1982) Nucl. Acids Res. 10, 5319-5332.
[12] Tran-Dinh, S. Neumann, J.M., Huynh-Dinh, T., Genissel, B., Igolen, J. and Simonot, G. (1982) Eur. J. Biochem. 124, 415-425.
[13] Patel, D.J. (1975) Biochemistry 14, 3984-3989.
[14] Patel, D.J. (1976) Biopolymers 15, 533-558.