A novel structural transition in poly(dG-Me^{5}dC): Z⇌B⇌Z

P.K. Latha and Samir K. Brahmachari*

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

Received 4 December 1984

Poly(dG-Me^{5}dC) is known to exhibit a B→Z transition in the presence of very high concentrations of NaCl. For the first time, we report the presence of a Z-structure in sodium concentrations as low as 0.5 mM. A novel Z⇄B⇄Z transition is observed as the salt concentration is gradually increased. The role of water structure in B to Z transitions is discussed.

Poly(dG-Me^{5}dC) Low salt Z-form Z⇄B⇄Z transition CD Ultraviolet spectroscopy

1. INTRODUCTION

The discovery of the left-handed Z-DNA in the crystals of d(CG)$_3$ [1] has initiated a series of studies on the factors responsible for B to Z transitions [2–6]. The B-form of DNA is believed to be the structure found in physiological salt conditions. The conformational change leading to the Z-form, first observed in poly(dG-dC) [7], takes place in the presence of molar quantities of NaCl. The midpoint of the B to Z transition for poly(dG-dC) is as high as 2.5 M NaCl or 0.7 M $MgCl_2$. These Z-structures are very well characterized by their inverted circular dichroism spectra [7], fibre diffraction pattern [8,9] and several other spectroscopic properties [10,11].

One of the most frequent modifications associated with gene inactivation is the methylation of cytosine residues on the C5 position in a d(CG) sequence. Methylation is reported to be associated with the control of transcription in vivo [12,13]. The crystal structure of (Me^{5}dC-dG)$_3$ shows that the methylation of cytosine destabilizes the B-form due to unfavourable interactions between the hydrophobic methyl groups and the water molecules, and stabilizes the Z-form through hydrophobic bonding [14]. Methylation of cytosine in poly(dG-Me^{5}dC) lowers the concentration of NaCl (700 mM) or $MgCl_2$ (0.6 mM) necessary to induce the B to Z transition [3].

In the light of these observations we have undertaken a detailed study of B to Z transitions in poly(dG-Me^{5}dC). Here we report a novel observation of the existence of Z-DNA in very low NaCl concentrations and a unique Z⇌B⇌Z transition as a function of salt. We have employed circular dichroism (CD) and UV absorption spectroscopy to characterize the Z-form, as these two techniques have been widely used in several of the studies to establish the presence of Z-DNA in polynucleotides and oligonucleotides [3,15,16].

2. MATERIALS AND METHODS

The sodium salt of poly(dG-Me^{5}dC) was obtained from Pharmacia-PL Biochemicals (lot no.782-26) and dialysed extensively against 0.5 mM sodium cacodylate buffer (pH 7.0) before use. A polynucleotide solution of A_{255nm} = 0.65–0.75 was used for all the experiments. The required salt concentration was obtained by the addition of small quantities of a very concentrated sodium chloride (analytical grade) solution or by the addition of solid sodium chloride. Calculations have been done after applying the necessary correction for dilution. The initial salt concentration was estimated by flame photometry. CD measurements were carried out using a Jasco J-20

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

spectropolarimeter with a temperature attachment. UV measurements were carried out using a Beckman DU 8B spectrophotometer. All the CD and UV studies were done at room temperature. Ellipticity values were calculated using an extinction coefficient of 7000 $M^{-1} \cdot cm^{-1}$ at 255 nm [17].

3. RESULTS

Fig.1 shows the CD spectra of poly(dG-Me^{5}dC) dialysed against 0.5 mM sodium cacodylate (pH 7.0) in the absence of NaCl, with 30 mM NaCl and 1.4 M NaCl. The respective UV spectra are given in fig.2a. Judging from the ellipticity at 290 and 255 nm, poly(dG-Me^{5}dC) exists in the Z-form both in the absence of NaCl and in 1.4 M NaCl, whereas for an intermediate salt concentration (30 mM) it has the B-conformation (fig.1). This observation is further supported by the presence of the characteristic shoulder in the UV spectra for the above Z-structures and its absence for the B-form (fig.2a).

The UV difference spectrum between the B- and Z-forms of poly(dG-Me^{5}dC) (220–320 nm region)

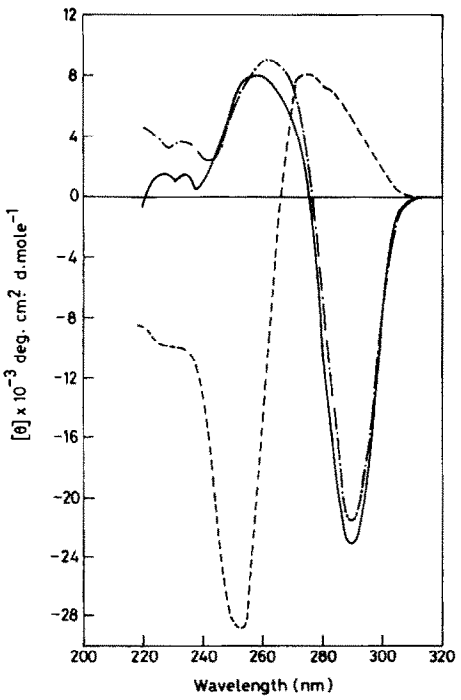

Fig.1. CD of poly(dG-Me^{5}dC). (——) No NaCl, (– – –) 30 mM NaCl, (-----) 1.4 M NaCl.

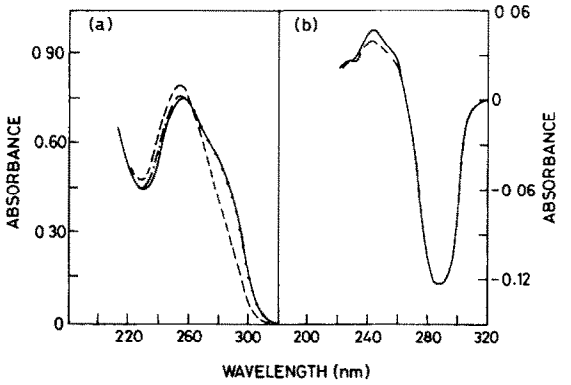

Fig.2. (a) UV spectra of poly(dG-Me^{5}dC). (——) No NaCl, (– – –) 30 mM NaCl, (-----)1.4 M NaCl. (b) Difference UV spectra between B- and Z-forms of poly(dG-Me^{5}dC). (——) 30 mM NaCl (B), no NaCl (Z); (– – –) 30 mM NaCl (B), 1.4 M NaCl (Z).

with 30 mM NaCl and in the absence of NaCl, respectively, is shown in fig.2b. This difference spectrum is the same as that obtained with the B- and Z-forms of the polynucleotide at 30 mM and 1.4 M NaCl, respectively. Both the difference spectra have a maximum around 295 nm as expected for a difference spectrum between B- and Z-forms [7]. The ratio of A_{260} to A_{295} for the low-salt and high-salt Z-forms are identical (~2.5), whereas for the B-form it is higher (~4.5).

Curve a of fig.3 gives the variation of molar ellipticity at 290 nm with the salt concentration for poly(dG-Me^{5}dC). For the sake of comparison, a similar plot of the degree of transition vs the NaCl concentration for poly(dG-dC) is given in the same figure (curve b). It can be seen from fig.3 that poly(dG-dC) exists in the B-form up to 2 M NaCl. On the other hand, for NaCl concentrations up to 15 mM, poly(dG-Me^{5}dC) exists completely in the Z-form and then undergoes a co-operative transition to the B-form with a mid-point of transition around 20 mM. Further, it continues to remain in the B-form for over a large range of NaCl concentrations. The second transition, to the Z-form, has a midpoint around 780 mM. This value is higher than the midpoint reported earlier for poly(dG-Me^{5}dC) (700 mM) [3]. However, such a variation is permissible as the dependence of these transitions on salt concentrations is found to differ from batch to batch due to variations in chain length [7]. A plot of $[\theta]_{255nm}$ against the NaCl concentration

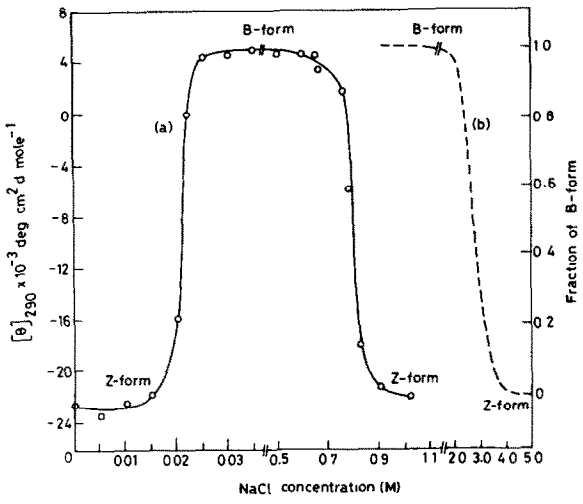

Fig.3. Curve a, variation of $[\theta]_{290\,nm}$ of poly(dG-Me^{5}dC) with NaCl concentration. Curve b, B⟶Z transition in poly(dG-dC) as a function of NaCl concentration; reconstituted from fig.5 of [7].

also gives similar results (fig.4).

4. DISCUSSION

The first evidence for a left-handed conformation came from the CD and UV studies of poly(dG-dC) [7]. That the alternating purine-pyrimidine sequences indeed exist in the left-handed form was confirmed by the crystal structure of d(CG)$_3$ [1]. Subsequent studies on poly(dG-dC) using laser Raman spectroscopy has proved beyond doubt that the high-salt form of poly(dG-dC) has a similar conformation to the crystal structure of d(CG)$_3$ [10]. Thus, the anomalous inversion in the CD spectrum of poly(dG-dC) in the high-salt solution was attributed to the left-handed Z-conformation. Since then, the inversion in the CD spectrum and the shoulder around 290 nm in the UV spectrum are taken to be the characteristic features of the Z-conformation of polynucleotides and oligonucleotides containing alternating purine-pyrimidine sequences [3,15,16,18].

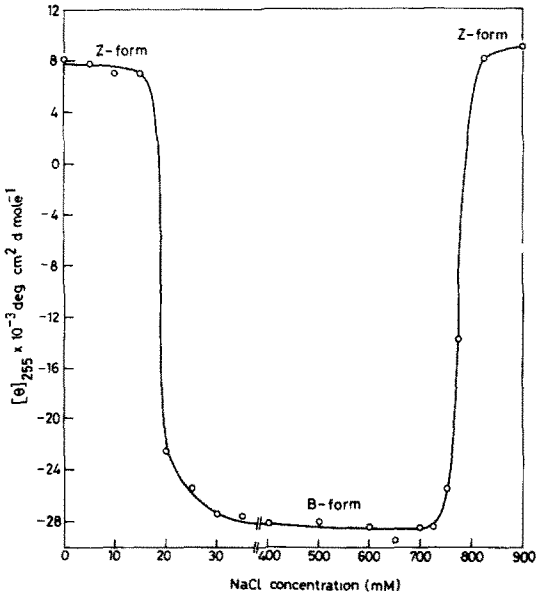

Fig.4. Variation $[\theta]_{255\,nm}$ of poly(dG-Me^{5}dC) with NaCl concentration.

The high-salt form of poly(dG-Me^{5}dC) is well characterized as the Z-form by several workers [3,19]. The low-salt structure of the same polynucleotide reported here has CD and UV spectra which are strikingly identical to those of the high-salt form. It is very unlikely that these two structures would be different. Thus, for the first time a Z-conformation is found to exist in such low concentrations of monovalent cation when the cytosine residues are methylated. The existence of a double transition like the Z⟷B⟷Z reported here is unique and has not been observed so far for any system. It may be pointed out that poly(dG-dC) dialysed against 0.5 mM sodium cacodylate buffer gives only a B-DNA CD spectrum.

It is generally believed that the B-form of DNA is stabilized by the ordered water structure around the DNA molecule. The evidence for this comes from the crystal structure of the dodecamer d(CGCGAATTCGCG) where there is a regular spine of water bridging the hydrophilic groups of alternate bases in the AT-rich region [20,21]. On the other hand, this backbone of water structure is disrupted by the 2-amino group of guanine suggesting that the guanine residues will destabilize the B-form [20]. It has also been shown that high concentrations of monovalent cations can effectively alter the water structure [22]. Hence the Z-form has been thought to be stabilized by salt by disturbing the water structure near the DNA molecule in the B-form. The stabilization of the Z-form of poly(dG-Me^{5}dC) in high-salt solution is further favoured by the hydrophobic interactions

of the methyl groups with the imidazole ring of guanine and the carbon atoms of the sugar [14]. These explanations hold good for the second transition (B to Z) reported here which takes place at a considerably higher salt concentration. However, it is difficult to explain the stabilization of the Z-form at such low NaCl concentrations as observed by us in terms of the decreased water activity around the DNA molecule. This clearly indicates that there are factors other than the low water activity which stabilize the Z-conformation.

It is interesting to note that the same factor, i.e., Na^+, which favours a structural transition up to a critical concentration, can reverse the effect at a higher concentration. Hence, any event that demands a Z to B transition can be brought about and reversed by the same Na^+. Thus, Na^+ can serve as an autoregulator for a Z to B transition of the DNA template containing alternating CG sequences. This effect is observed only when cytosine residues are methylated. Thus, it is tempting to suggest that methylation in conjunction with fluctuations in Na^+ concentration at the millimolar level may bring about a reversible B to Z transition in small stretches of alternating CG sequences in natural DNA. These stretches could probably then serve as sites for regulation.

ACKNOWLEDGEMENTS

The authors thank Professor V. Sasisekharan for critically reading the manuscript. Financial support from DST, India (MBU/DST/VS/44) and UNESCO/UNDP is gratefully acknowledged.

REFERENCES

[1] Wang, A.H.-J., Quigley, G.J., Kolpak, F.J., Crawford, J.L., Van Boom, J.H., Van der Marel, G. and Rich, A. (1979) Nature 282, 680–686.
[2] Klysik, J., Stirdivant, S.M., Larson, J.E., Hart, P.A. and Wells, R.D. (1981) Nature 290, 672–677.
[3] Behe, M. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sci. USA 78, 1619–1623.
[4] Ramesh, N. and Brahmachari, S.K. (1983) FEBS Lett. 164, 33–37.
[5] Moller, A., Nordheim, A., Kozlowski, S.A., Patel, D.J. and Rich, A. (1984) Biochemistry 23, 54–62.
[6] Latha, P.K., Majumder, K. and Brahmachari, S.K. (1983) Curr. Sci. 52, 907–910.
[7] Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375–396.
[8] Sasisekharan, V. and Brahmachari, S.K. (1981) Curr. Sci. 50, 10–13.
[9] Arnott, S., Chandrasekhar, R., Birdsall, D.L., Leslie, A.G.W. and Ratliff, R.L. (1980) Nature 283, 743–745.
[10] Thamann, T.J., Lord, R.C., Wang, A.H.-J. and Rich, A. (1981) Nucleic Acids Res. 9, 5443–5457.
[11] Patel, D.J., Canuel, L.L. and Pohl, F.M. (1979) Proc. Natl. Acad. Sci. USA 76, 2508–2511.
[12] Razin, A. and Riggs, A.D. (1980) Science 210, 604–610.
[13] Ehrlich, M. and Wang, R.Y.-H. (1981) Science 212, 1350–1357.
[14] Fujii, S., Wang, R.Y.-H., Van der Marel, G., Van Boom, J.H. and Rich, A. (1982) Nucleic Acids Res. 10, 7879–7892.
[15] Zacharias, W., Larson, J.E., Klysik, J., Stirdivant, S.M. and Wells, R.D. (1982) J. Biol. Chem. 257, 2775–2782.
[16] Quadrifoglio, F., Manzini, G. and Yathindra, N. (1984) J. Mol. Biol. 175, 419–423.
[17] Gill, J.E., Maarimas, J.A. and Bishop, C.C. jr (1974) Biochim. Biophys. Acta 335, 330–348.
[18] McIntosh, L.P., Griegar, I., Eckstein, F., Zarling, D.A., Van de Sande, J.H. and Jovin, T.M. (1983) Nature 304, 83–86.
[19] Chen, C., Cohen, J.S. and Behe, H. (1983) Biochemistry 22, 2136–2142.
[20] Wing, R., Drew, H.R., Takano, T., Broka, C., Tanaka, S., Itakura, K. and Dickerson, R.E. (1980) Nature 287, 755–758.
[21] Drew, H.R. and Dickerson, R.E. (1981) J. Mol. Biol. 151, 535–556.
[22] Bonner, O.D. and Jumper, C.F. (1973) Infrared Phys. 13. 233–242.