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New DNA polymorphism: evidence for a low salt, left-handed form of poly(dG-m^{5}dC)

Burt G.Feuerstein[1+], Laurence J.Marton[1,2], Max A.Keniry[3], Donald L.Wade[3] and Richard H.Shafer[3*]

[1]Brain Tumor Research Center, [2]Department of Laboratory Medicine, School of Medicine, and [3]Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA 94143, USA

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ABSTRACT

Spectroscopic studies on solutions of poly(dG-m^{5}dC) over a wide range of salt concentration are presented. Low salt solutions ([Na^+]) $\leq$ 2 mM) of poly(dG-m^{5}dC) produce circular dichroism (CD) spectra typical of the left-handed, Z form at high salt ([Na^+] = 1.75 M). Solutions of poly(dG-m^{5}dC) at intermediate salt concentrations, e.g., 142 mM, yield CD spectra characteristic of the right-handed, B conformation. ^{31}P NMR spectra of the low salt form of poly(dG-m^{5}dC) reveal two well separated peaks, split by 1.4 ppm, consistent with a dinucleotide repeat. Kinetic studies show that the transition from the low salt form to the right-handed B form is slow, as expected for a major conformational change. These results suggest that the Z conformation in poly(dG-m^{5}dC) can be stabilized at very low salt as well as at high salt.

INTRODUCTION

The confirmation by X-ray crystal studies (1,2) of a left-handed form of poly(dG-dC) originally suggested by the work of Pohl and Jovin (3) has been a significant development in the understanding of nucleic acid structure (4,5). Recent experiments point to the likely biological importance of this flexibility in DNA conformation in terms of gene regulation (4,5). Various combinations of conditions and polynucleotides have been shown to result in a left-handed form (3-6). In the case of poly(dG-dC) and poly(dG-m^{5}dC), such conditions are either strongly dehydrating, involving high salt concentrations or addition of alcohol, or require the presence of oligovalent cations such as spermine (7). Furthermore, temperature also appears to play a role in the B to Z transition of both poly(dG-dC) and poly(dG-m^{5}dC) (8,9).

Wu and Behe (10) have recently described a low salt, Z conformation in the synthetic polymer poly(rG-dC). Upon increasing the salt concentration, this polymer undergoes a transition first to the A form and then to the Z form again. The conformation that is stable at intermediate salt is the A form, presumably due to the presence of an alternating ribose-deoxyribose sugar backbone. In the experiments described below, we present evidence for a left-

handed form of the DNA polymer poly(dG-m^5dC) stable at very low salt concentrations in the absence of oligovalent cations. Increasing concentrations of NaCl lead first to the B conformation and then to the Z conformation. This unexpected result may have profound implications for our understanding of the forces that stabilize the various forms of DNA and adds another dimension to the possible biological role of DNA polymorphism.

MATERIALS AND METHODS

Poly(dG-m^5dC) was obtained from Pharmacia-P.L. Biochemicals. Concentrations of poly(dG-m^5dC) were determined spectroscopically using a molar (base pair) extinction coefficient, ε_{260}, of 14,200. When first dissolving the polynucleotide, the sample was typically annealed at 50°C for 10 minutes. Samples for UV and CD (circular dichroism) studies were prepared by dialysis against low salt buffers (0.5 or 2 mM Na-cacodylate) containing 0.1 mM Na_2EDTA. In some cases, stock solutions of poly(dG-m^5dC) were exhaustively dialyzed against 2 M NaCl, 1 mM Na_2EDTA, then dialyzed back down to a low salt buffer. Samples for NMR studies were prepared by sonicating poly(dG-m^5dC) in 0.5 mM Na-cacodylate, 1 M NaCl for 3 hr under CO_2 in an ice-ethanol bath, followed by extensive dialysis first against 20 mM NH_4HCO_3, then against 5 mM NH_4HCO_3 (and 1 mM EGTA in each case) and lyophilization. The low salt sample was made by resuspending the lyophilized polynucleotide in a buffer consisting of 0.5 mM Na-cacodylate, 0.1 mM Na_2EDTA, 20% D_2O, pH meter reading 7.4. The intermediate salt solution was made by resuspending in H_2O containing 50 mM NaCl and 20% D_2O, pH meter reading 7.5. Reproducible results were obtained on freshly prepared solutions or those stored at 4°C or -20°C for several days. Older solutions sometimes exhibited right-handed CD spectra under low salt conditions.

UV spectra were recorded on a Cary 118 spectrophotometer while CD spectra were obtained on a JASCO 500A spectropolarimeter. ^{31}P NMR spectra were obtained on a home built multinuclear spectrometer operating at 240 MHz for protons at the UCSF School of Pharmacy NMR Facility. Spectra were recorded at 45°C with quadrature detection, a sweep width of ± 630 Hz, a 65° pulse, a recycle time of 3.3s, 2W proton decoupling power and with 4K data points collected in each channel. Chemical shifts are presented relative to external trimethyl phosphate. 8000-9000 scans were accumulated and 25 Hz linebroadening was applied to each spectrum.

The kinetics of the transition from both the low salt and high salt left-handed conformations to the intermediate salt, B conformation were

investigated as follows. A solution of poly(dG-m^5dC) in 2 mM Na-cacodylate was mixed by hand with a solution of NaCl in the same buffer to yield a final NaCl concentration of 0.2 M. The CD amplitude at 292 nm was then monitored as a function of time after mixing. A similar experiment was then carried out starting with poly(dG-m^5dC) in 2 mM Na-cacodylate and 1.75 M NaCl which was then mixed with enough buffer alone to bring the final NaCl concentration down to 0.2 M. In order to observe the bulk of the transition using hand mixing, these experiments were carried out at 16°C.

RESULTS

The circular dichroism spectra of solutions of poly(dG-m^5dC) in low, medium and high salt solutions ([Na^+]=2 mM, 142 mM, and 1.75 M) in 2 mM Na-cacodylate are presented in Figure 1a. The high salt conditions produce an inverted spectrum typical of the Z form of this polymer. Intermediate salt conditions show a spectral pattern typical of the B form. Surprisingly, the low salt form displays a spectrum very similar to that of the high salt form, indicative of a left-handed conformation. Low concentrations of such multivalent cations as spermine and Mg^{++} have been previously shown to induce the Z form in poly(dG-m^5dC) (6). However, a conformational change in poly(dG-m^5dC) at low sodium concentration in the absence of such multivalent cations has not been described previously.

Figure 1b shows the results of a NaCl titration of the low salt form. The transition towards the right-handed form exhibits an isobestic point at 273 nm and occurs over a very narrow range of concentration, typical of a cooperative change in conformation (see Figure 1c). Thus, as the Na^+ concentration increases from 2 mM or less, the CD spectrum changes from left-handed to right-handed and then back to left-handed. This non-monotonic response of DNA to salt has never been reported before.

While the data shown in Figure 1a,b and c refer to solutions of poly(dG-m^5dC) in 2 mM Na-cacodylate, we have obtained the same CD spectrum using buffers containing EDTA (0.5 mM Na-cacodylate, 0.1 mM Na_2EDTA, pH 7.2; 0.4 mM Na-phosphate, 0.08 mM Na_2EDTA, pH 7.4). Figure 1d shows the effect of adding NaCl to a solution of poly(dG-m^5dC) in 0.5 mM Na-cacodylate, 0.1 mM Na_2EDTA, pH 7.4 at 37°C. A sharp transition is observed, as in Figure 1c, although it is shifted to higher NaCl concentrations. Thus, the Z-like CD spectrum observed in low salt solutions of poly(dG-m^5dC) is not due to trace levels of undesired cations.

Additional characterization was carried out using ^{31}P NMR. It is well

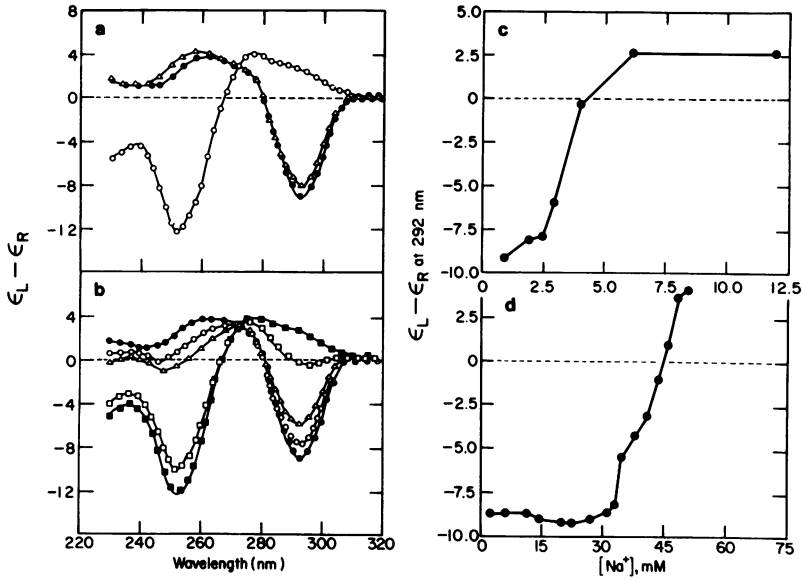

Fig. 1. Circular dichroism spectra of solutions of poly(dG-m^5dC) in Na-cacodylate buffer with various concentrations of NaCl. Solutions in a, b and c were made from a stock solution that had been exhaustively dialyzed against 2 M NaCl and 1 mM Na_2EDTA. (a) poly(dG-m^5dC), in 2 mM Na-cacodylate, pH 7.4, 25°C, concentration in base pairs, and the added NaCl concentration are, respectively: 7 μM, OM (●); 6 μM, 0.14 M (○); 4 μM, 1.75 M (△). (b) titration of 7 μM poly(dG-m^5dC), in 2 mM Na-cacodylate, pH 7.4, 25°C, with the following concentrations of added NaCl: OM (●); 0.05 mM (○); 1 mM (△); 2.1 mM (□); 4.1 mM (■). (c) CD amplitude at 292 nm as a function of total [Na^+] at 25°C. Middle five points replotted from part b. First point from 0.8 mM Na-cacodylate solution, last point from 2 mM Na-cacodylate solution with 10 mM NaCl. (d) CD amplitude at 292 as a function of total [Na^+] at 37°C in 0.5 mM Na-cacodylate, 0.1 mM Na_2EDTA, pH 7.2, with 12 μM poly(dG-m^5dC) and increasing amounts of NaCl.

established that the B form of poly(dG-dC) yields a single peak (11,12), consistent with a mononucleotide repeat unit, and that the B form of poly(dG-m^5dC) gives a partially resolved doublet suggestive of a dinucleotide repeat (13). The Z form of these polynucleotides shows two well separated peaks due to the fundamental repeating unit being a dinucleotide, with distinctly different phosphate torsion angles (11-13). The ^{31}P NMR spectra of the low salt form (in the presence of EDTA) and intermediate salt form of poly(dG-m^5dC) are displayed in Figure 2. In the spectrum of the low salt sample, there are two peaks separated by 1.4 ppm, which suggests again a dinucleotide repeat for the low salt, left-handed conformation of poly(dG-m^5dC).

The kinetics of the transitions in poly(dG-m^5dC) from both the high salt

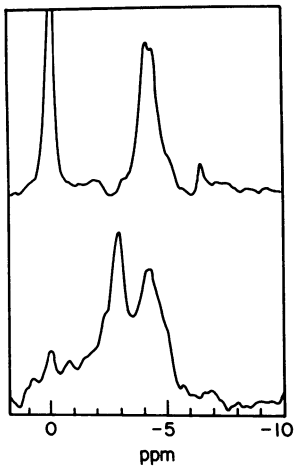

Fig. 2. 97.6 MHz ^{31}P NMR spectra of poly(dG-m^{5}dC). Top: sample in 50 mM NaCl at a concentration of 4 OD/ml in a 10 mm tube. Chemical shift of peak is -4.3 ppm. Bottom: Sample in 0.5 mM Na-cacodylate, 0.1 mM Na_2EDTA, pH 7.2 at a concentration of 28 OD/ml in a 5 mmm tube. Chemical shifts are -2.95 and -4.35 ppm. See text for details.

and the low salt forms to the same intermediate salt conditions were examined using salt-jump techniques. The results, shown in Figure 3, demonstrate that the transition from the low salt form occurs more slowly than that from the high salt form, supporting the notion that a major conformational change has occurred under both sets of conditions. Furthermore, the intermediate salt solution can be either jumped or dialyzed back to the low salt conditions with

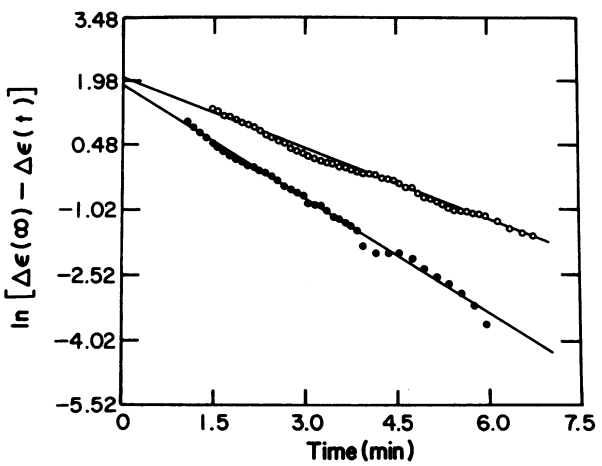

Fig. 3. Salt-jump relaxation kinetics of 2 mM Na-cacodylate solutions of poly(dG-m^{5}dC), 7 μM base pairs, pH 7.4 at 16°C, starting from either no additional NaCl (0) or 1.75 M NaCl (●). Final conditions in each case were 2 mM Na-cacodylate and 0.2 M NaCl. Smooth line represents a single exponential fit with a rate constant of $9.1 \times 10^{-3} s^{-1}$, (0), and $1.5 \times 10^{-2} s^{-1}$, (●).

the reappearance of the inverted CD spectrum. Thus, this transition is completely reversible.

It is of interest to note several other aspects of the low salt transition in poly(dG-m^5dC). There appears to be a concentration dependence in that higher polymer concentrations shift the equilibrium toward the right-handed B conformation. Also, there is a temperature dependence such that increased temperature favors the left-handed conformation. This can be seen in the low salt transitions displayed in Figure 1c and d. The higher temperature (45°C) was needed to obtain a Z-like ^{31}P NMR spectrum in Figure 2 at higher polymer concentrations (2 mM base pair). A similar temperature dependence in the B to Z transition for poly(dG-m^5dC) in the presence of Mg^{++} or high salt has been reported previously by Roy and Miles (8) and by Behe et al. (9).

DISCUSSION

The experiments we have described above demonstrate the existence of a left-handed conformation of poly(dG-m^5dC) under very low salt conditions in the absence of any oligovalent cations. Furthermore, we have shown that this leads to a cooperative transition from this conformation to the right-handed B conformation of poly(dG-m^5dC) upon small increases in NaCl concentration. Additional increases in salt concentration result in the well characterized B to Z transition for this polymer. This behavior has never been reported before for a DNA polynucleotide.

There have been several recent studies on poly(dG-m^5dC) that have described results that differ from those presented here. Thomas and Bloomfield (14) have indicated that poly(dG-m^5dC) shows a left-handed CD spectrum in 1 mM Na-cacodylate, 1 mM NaCl at 4°C, but when EDTA (0.15 mM) was added, the spectrum reverted to that of right-handed DNA. We have found the left-handed form to remain stable in the presence of EDTA at or above room temperature. As mentioned earlier, we have observed a strong temperature dependence for the low salt, left-handed to right-handed transition, with higher temperature favoring the left-handed form. The lower temperature used in the work of Thomas and Bloomfield (14) compared to the higher temperature used in our studies may explain these differences.

Chen et al. (7) have described studies on poly(dG-m^5dC) in very low salt buffers such as 0.5 mM Na-cacodylate, 20 μM Na_2EDTA in which the polynucleotide conformation was right-handed in the absence of oligovalent cations. As indicated above, our results were only obtained in a reproducible

fashion if freshly prepared samples were used. This is a possible source of the discrepancy in our results.

It is of interest to compare our results with those of Wu and Behe (10) on the Z-A-Z transition in poly(rG-dC). In the poly(dG-m^5dC) system, the low salt and high salt forms exhibit a CD spectrum that is essentially an inverted form of the spectrum exhibited by the intermediate salt form. This is not the case with poly(rG-dC) due to the fact that the intermediate salt form is the A conformation whose CD spectrum is very different from that of the B form. The low salt transition in poly(dG-m^5dC) is highly cooperative, as shown in Figure 1, while the Z-A transition in poly(rG-dC) is non-cooperative. This is also in contrast to the results of Ivanov and Minyat (15) who have shown that the Z-A transition is cooperative in poly(dG-dC). Finally, in the ^{31}P NMR spectrum of poly(rG-dC) at low salt, the upfield peak appears to be split. Wu and Behe suggested that this may reflect a mixture of Z_I and Z_{II} conformations in poly(rG-dC). Such considerations may also apply to poly(dG-m^5dC), although further work is needed in both cases to confirm this hypothesis.

The inverted CD spectrum with an isobestic point in common with the high salt form, the well separated doublet in the ^{31}P NMR spectrum, and the similarity in the UV spectra of the high and low salt forms (data not shown) all strongly suggest that this low salt form of poly(dG-m^5dC) resembles the Z form. A comparison of the crystal structure of the left-handed Z (1,2) and right-handed B (16) forms shows two electrostatic effects to be considered. First, the phosphate-phosphate distance of closest approach is considerably shorter in the Z form than in the B form. This would tend to favor the Z form at high salt and the B form at low salt. Second, the linear charge density along the helix axis is greater for the B form than the Z form, and this would favor the Z conformation at low salt. There is no simple way to predict the net effect of these two opposing trends.

We note that Soumpasis (17) has successfully predicted the high salt B to Z_I transition for a dC-dG dodecamer with a statistical mechanics calculation of the interaction between the DNA phosphates and the diffuse cloud of surrounding ions. In addition, Soumpasis noted that in the range of 0.4 to 1.8 M monovalent cation concentration the Z_{II} form is only slightly less favorable thermodynamically than the B form. This notion has been elaborated by Soumpasis et al. (18) who calculate a B-Z_{II} transition at 0.24 M monovalent salt by the same approach. It is possible that at still lower salt concentrations, the Z_{II} form actually becomes more stable than the B form. This behavior would most likely be facilitated by the presence of the methyl

group on cytosine, which is not included in these purely electrostatic calculations. High resolution ^{1}H NMR studies are underway to determine to what extent the low salt conformation of poly(dG-m^5dC) resembles the Z form.

A non-monotonic response of DNA conformation to salt concentration could have significant biological implications. Methylated sequences are present in naturally occurring DNA and have been related to regulation of gene expression (19). The ability to convert DNA from the right-handed B form to a left-handed form by either increasing or decreasing ion concentration could provide a sensitive means of controlling various DNA functions by virtue of stabilizing a specific conformation under a very narrow range of conditions. In any case, the ability to alter drastically the state of dG-m^5dC sequences at either high or low salt adds a new dimension to the issue of environmental balance and DNA polymorphism.

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+Present address: Department of Pediatrics, School of Medicine, University of California, San Francisco, Ca 94143.

*To whom correspondence should be addressed

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. Drew, H., Takano, T., Tanaka, S., Itakura, K., and Dickerson R.E. (1980) Nature 286, 567-573.
3. Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375-396.
4. Jovin, T.M., McIntosh, L.P., Arndt-Jovin, D.J., Zarling, D.A., Robert-Nicoud, M., van de Sande, J.H., Jorgenson, K.F., and Eckstein, F.J. (1983) J. Biomol. Str. Dyns. 1, 21-57.
5. Rich, A., Nordheim, A., and Wang, A.H.-H. (1984) Ann. Rev. Biochem. 53, 791-846.
6. Behe, M. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sci. USA 78, 1619-1623.
7. Chen, H.H., Behe, M.J., and Rau, D.C. (1984) Nucl. Acids Res. 12, 2381-2389.
8. Roy, R.B. and Miles, H.T. (1983) Biochem. Biophys. Res. Comm. 115, 100-105.
9. Behe, M.J., Felsenfeld, G., Szu, S.L., and Charney, E. (1985) Biopolymers 24, 289-300.

10. Wu, H.-Y. and Behe, M.J. (1984) Proc. Natl. Acad. Sci. USA 81, 7284-7287.
11. Patel, D.J., Canuel, I.L., and Pohl, F.M. (1979) Proc. Natl. Acad. Sci. USA 76, 2508-2511.
12. Patel, D.J., Kozlowski, S.A., Nordheim, A., and Rich, A. (1982) Proc. Natl. Acad. Sci. USA 79, 1413-1417.
13. Chen, C.-W., Cohen, J.S., and Behe, M. (1983) Biochemistry 22, 2136-2142.
14. Thomas, T.J. and Bloomfield, V.A. (1985) Biochemistry 24, 713-719.
15. Ivanov, V.I. and Minyat, E.E. (1981) Nucleic Acid Res. 9, 4783-4799.
16. Wing, R., Drew, H., Takano, T., Broka, C., Tanaka, S., Itakura, K., and Dickerson, R.E. (1980) Nature 287, 755-758.
17. Soumpasis, D.M. (1984) Proc. Natl. Acad. Sci. USA 81, 5116-5120.
18. Soumpasis, D.M., Wiechen, J., and Jovin, T.M., submitted.
19. Razin, A. and Riggs A.D. (1980) Science 210, 604-610.