Alteration of baroreceptor discharge following NE or VP administration may depend upon the relationship of the baroreceptor endings to smooth muscle cells within the adventitia[10]. The baroreceptor endings have been described as being located either in series or in parallel with the muscle elements. Single fiber recordings after either NE or VP showed that most fibers increased their discharge frequency either at normal pulsatile pressures or at perfused sinus pressures between 5.2 kPa and 23.4 kPa; presumably receptors located in series with contacting smooth muscle cells. A few single fibers showed a decreased discharge frequency with the sinus at normal pulsatile pressure and at all perfused sinus pressures; presumably located in parallel with smooth muscle cells. Similar results were obtained by Bergel et al.[8] in a few baroreceptor fibers in the dog. Since both NE and VP cause contraction of smooth muscle similar effects would be expected on nerve endings. Other workers[7] have suggested that 'sensitization' of the baroreceptor endings by NE could account for an increased baroreceptor discharge. This conclusion was based on the fact that 10^{-9} M NE increased discharge but 10^{6} M NE did not. They postulated that contraction of the vessel wall unloaded the baroreceptors in the presence of 10^{-6} M NE. If NE had sensitized receptors and VP had not, it may have been possible to show different effects. As we were unable to show any differences between NE and VP we must conclude either that both or neither substance causes sensitization. The fact that some fibers decreased their discharge to either NE or VP is consistent with the view that the receptor activity is modulated by smooth muscle activity rather than by sensitization of the receptors.

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Conformational flexibility of poly (dG-m^{5}dC) under very low salt conditions

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Summary. The methylated DNA polymer poly (dG-m^{5}dC) which exhibits a B helical conformation in solutions containing 20 mM NaCl, undergoes a gradual and reversible transition to the Z conformation as the NaCl concentration is lowered. The midpoint of this transition occurs around 5–6 mM NaCl. The conformational flexibility of this polymer at such low NaCl concentrations opens up the possibility of studying the effects of other perturbants with negligible interference from salt concentration effects.

The conformational transitions of the methylated polymer poly (dG-m^{5}dC) have received much attention in recent years, since the dinucleotide sequence dG-m^{5}dC occurs very frequently in eukaryotic DNA[1]. Possibilities of the correlation of such methylations with the control of genetic expression have been explored[2]. It has been shown by Behe and Felsenfeld[3] that the methylated polymer undergoes the B to Z transition very similar to the unmethylated polymer, but at much lower salt concentrations, with transition mid points occurring near 0.7 M NaCl or at 0.6 mM $MgCl_2$ in presence of 50 mM NACl, as opposed to the free polymer which has a transition mid point at 2.5 M NaCl or 0.7 $MgCl_2$[4]. These transitions of the methylated polymer have been recently confirmed by ^{31}P, ^{13}C and ^{1}H NMR studies[5–7] which indicate that the B to Z transition in the methylated polymer is complete at around 1.5 M NaCl. In the present study, we have detected a second B to Z transition of this methylated polymer at much lower NaCl concentrations below 20 mM in the absence of other cations. The transition is quite reversible in the range of 20 mM to 2 mM NaCl.

Materials and methods. The alternating double stranded polymer poly (dG-m^{5}dC) was purchased from P.L. Biochemicals. The polymer was homogeneous in CsCl density gradient with an $S_{20,w}$ of 8.5. Stock solutions of the polymer were prepared in 0.2 M NaCl solutions made from glass distilled, deionized water. The stock solutions ranged in pH from 6.8 to 7.0. Appropriate volumes of the stock solution were diluted freshly to obtain the required NaCl concentrations. Solid NaCl was added to gradually increase the concentration from 2 mM to 20 mM during the reversibility studies. All circular dichroic (CD) spectra were taken in a Jasco 10 Spectropolarimeter in a temperature controlled, jacketted cylindrical cell.

Results and discussion. Figure 1 represents the CD spectra of the methylated polymer at different NaCl concentrations ranging from 20 mM to 1 mM. At NaCl concentrations of 20 mM and above, this polymer exhibits a CD spectrum indicative of B helical conformation, with a broad positive band centering at 290 nm and a sharp negative band at 252 nm. As the concentration of NaCl is gradually lowered, an inversion of the CD spectrum takes place with a sharp negative band at 293 nm and a positive band at 276 nm. The variations of the CD ellipticities at different ionic strengths ranging from 2 mM to 30 mM NaCl are shown in figure 2. The negative ellipticity at 252 mM shows a sharp cooperative type of transition while those at 275 and 293 nm are rather broad and show biphasic tendencies. The transition midpoints in each case center around 5–6 mM NaCl. Under similar salt concentration conditions, the unmethylated polymer poly d(G-C) did not show any conformational transitions at room temperature and remained in the B helical conformation even at 2 mM NaCl[8].

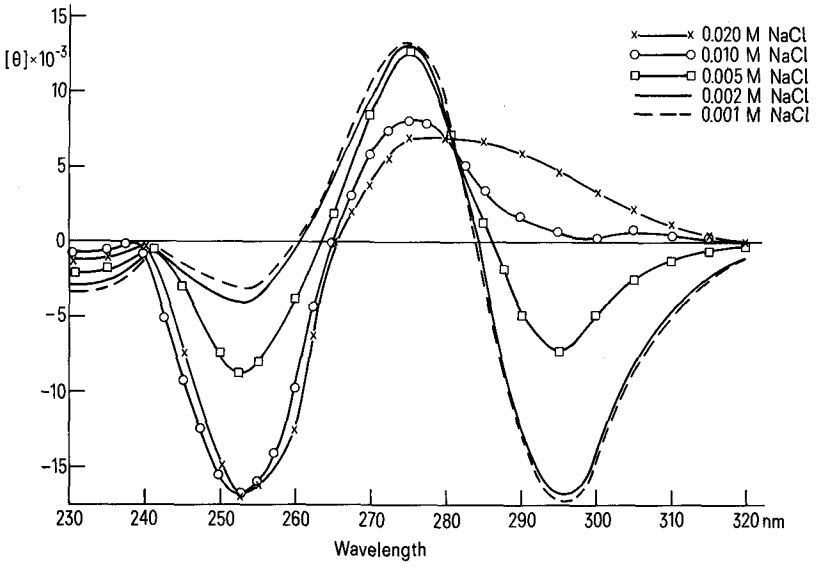

Figure 1. CD spectra of poly (dG-m^{5}dC) in solutions of NaCl concentration ranging from 20 mM to 1 mM.

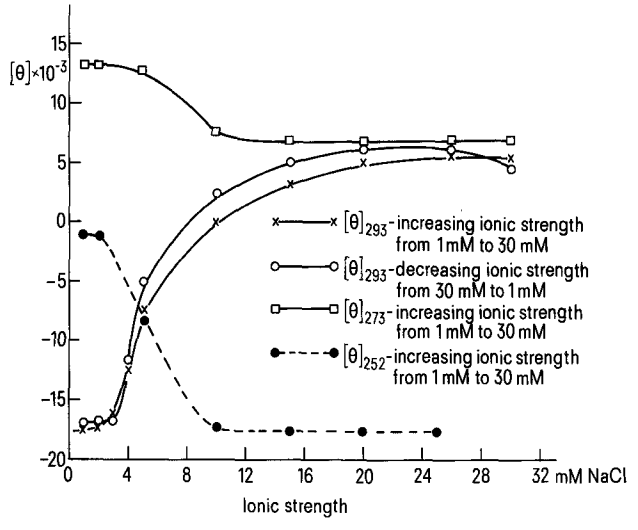

Figure 2. Variation of CD ellipticities of poly (dG-m^{5}dC) with NaCl concentrations.

The origin of the B-Z transition in the methylated polymers have been speculated on in many recent reports. The X-ray diffraction studies of the unmethylated polymer as well as the methylated polymer[9] show a close packing pattern of the bases in the Z form (13 ± 1 base pairs per turn) compared to the B form (10 ± 1 base pairs per turn), with the phospho diester negative charges being closer to each other in the Z form. In addition, the guanine residues assume the 'syn' rotational conformation in the Z form as opposed to the 'anti' conformation in the B form[10]. The B-Z transitions observed in these polymers on increasing the salt concentrations have been hypothesized to be due to the neutralization of the closely situated negative charges of the phosphates by the cations of the salts. However, Behe and Felsenfeld[3] indicate that there is no simple correlation between the cationic charges and the ability to induce the B-Z transition, at least in the methylated polymer. Our present results on the occurrence of the B-Z transitions even at salt concentrations as low as 5–6 mM NaCl seem to support such a lack of correlation between the cationic charges and stability of the Z conformation. Recent quantum chemical studies of the B-Z transitions of the methylated polymers[11] indicate that in the B form, the hydrophobic 5-methyl group of cytosine is located in the major groove, exposed to the solvent whereas in the Z form, the methyl group is situated in a more shielded hydrophobic environment. At very low concentrations of salt, the solvent effects will be felt more on the DNA polymer and hence it may explain the ease of transition of the methylated polymer to the Z form even at very low salt conditions as reported here in our present studies. The advantages of using such low salt conditions to induce the B-Z transition in the methylated polymer lie in the fact that since the transition is easily reversible, it is possible to fix the molecule in any intermediate conformation and the effects of other perturbants in shifting the transition towards either direction can be studied easily[12] since any conformational effects caused under these low salt conditions will be solely due to the perturbant alone. In addition, the observing of NMR signals becomes much easier under these low salt conditions compared to the very high salt conditions required for the unmethylated polymer. Further investigations of the very low salt transitions of this and other DNA polymers are currently in progress.

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