Sequence dependence and role of 5'-phosphate in the B to Z transition

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Spectroscopic studies on pd(CG)$_3$ and pd(GC)$_3$ have been carried out to elucidate the sequence dependence and effect of free 5'-phosphate on the B to Z transition. Unlike d(CG)$_3$, pd(CG)$_3$ fails to undergo salt-induced B to Z transition at ambient temperature. Model building studies have been carried out to determine the inhibitory role of the 5'-phosphate group, but have been unsuccessful.

Sequence dependence   *Deoxyoligonucleotide*   *B→Z transition*   *5'-Phosphate effect*   *Circular dichroism*
Ultraviolet difference spectroscopy

1. INTRODUCTION

Identification of the inherent conformational flexibility of a polynucleotide backbone [1,2] and the recent observation of the structural variation in single crystals of small deoxyoligonucleotides [3–6] have revived interest in the conformational variations of DNA. The discovery of the left-handed Z-DNA conformation in single crystals of d(CG)$_3$ [5] has led several investigators to study the structural transition of the right-handed B-form to the left-handed Z-form in alternating purine-pyrimidine sequences [7–9]. Recent studies have clearly demonstrated that various factors such as dehydrating solvents [8], cations [10,11], temperature [12], topological strain [13] and base modifications like methylation [11,14] or bromination [15] can effectively stabilize the Z-DNA conformation in polynucleotides with alternating CG sequences. In natural systems such alternating CG stretches are of rather smaller length. Therefore, study of B to Z transitions at the oligomer level is imperative. X-ray diffraction analysis of d(CG)$_3$ [5] and theoretical studies of poly(dG-dC) in the Z-form [16] have shown that the stacking overlap between G and C in the d(GpC) is different from that between C and G in the d(CpG). It is quite likely that the extra phosphate at the 5'-end may influence the charge density of the molecule which in turn can affect the stacking overlap of the base-pairs. We have studied the effect of the presence of a free 5'-phosphate group on the conformation of pd(CG)$_3$ and pd(GC)$_3$ with special reference to the B to Z interconversion. We find that the incorporation of a 5'-phosphate group in d(CG)$_3$ inhibits the B $\longrightarrow$ Z transition in high salt solution.

2. MATERIALS AND METHODS

pd(CG)$_3$ and pd(GC)$_3$ were purchased from PL-Biochemicals. As the oligomers were found to be pure by urea-PAGE, these were used without further purification. Sodium cacodylate was from Sigma (USA). All other reagents were of analytical grade. Both oligomers were dissolved in low salt buffer (2 mM sodium cacodylate, 0.1 mM EDTA, pH 7.5). Circular dichroism (CD) spectra were recorded on a Jasco J20 spectropolarimeter fitted with a temperature accessory and an MLS constant temperature circulating bath (GDR). The spectra reported are averages of several scans. Constant temperature was obtained by prolonged equilibration. UV spectra were recorded on a Beckman DU 8B spectrophotometer. Models were built using the HGS-DNA model kit.

3. RESULTS

Fig.1 shows the CD spectra of pd(CG)₃ at 0 and 60°C and of pd(GC)₃ at 0°C in low salt solution. Both oligomers exhibit characteristic B-form spectra under this condition at low temperature. The presence of an ordered structure at lower temperature is well documented by the observed melting of the duplex as a function of temperature (inset to fig.1). The two oligomers exhibit similar CD melting patterns in 2 mM cacodylate buffer. The mid-point of the transition for both was found to be 38.5 ± 0.5°C.

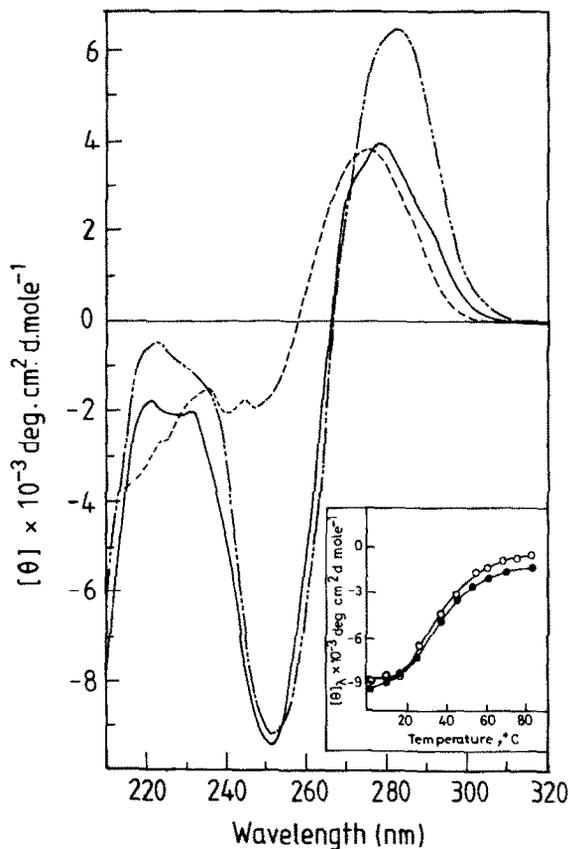In the presence of 5 M NaCl, d(CG)₃ exhibits inverted CD spectra with a characteristic negative band at ~295 nm [17] and has been shown to exist in the left-handed Z-form [18]. To determine the effect of the 5'-phosphate group on the B ⟶ Z transition we have compared the CD spectra of pd(CG)₃ and d(CG)₃ in 5 M NaCl (fig.2). It is interesting to note that at 25°C pd(CG)₃ remains in the B-form whereas d(CG)₃ undergoes a B to Z transition [17]. However, on cooling to 0°C pd(CG)₃ exhibits a weak negative band at ~295 nm. Such negative bands at ~295 and 296–300 nm have been observed in the case of the Z- and A-forms, respectively [9,21]. However, while many d(CG) oligomers undergo NaCl-induced B ⟶ Z transition [17], the B ⟶ A transition of d(CG) oligomers has not been observed
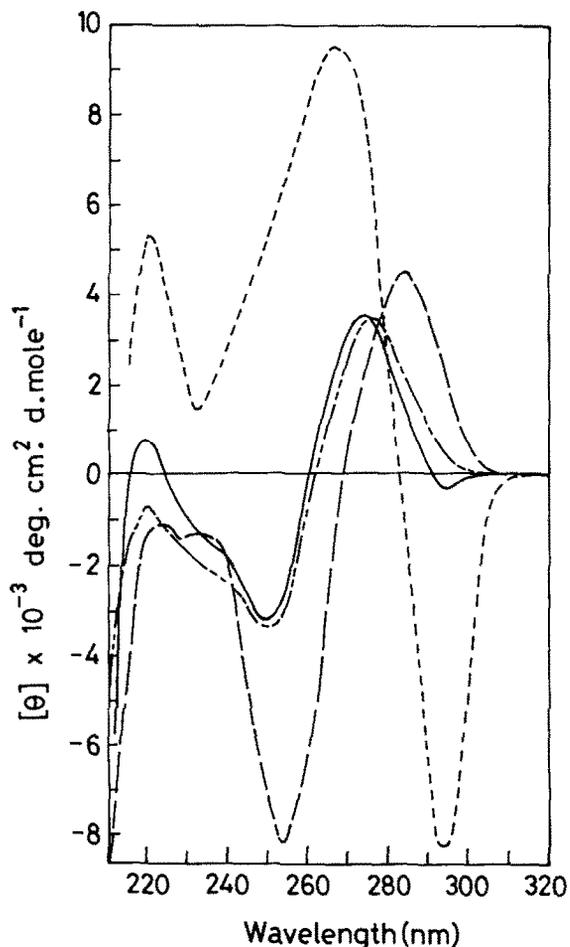

Fig.1. CD spectra of pd(CG)₃ and pd(GC)₃ in low salt buffer. pd(CG)₃: at 0°C (——) and 60°C (———). pd(GC)₃: at 0°C (———). Inset: temperature vs [θ]λ plot for pd(CG)₃ (λ = 250 nm) (●——●) and pd(GC)₃ (λ = 254 nm) (○——○).

Fig.2. CD spectra of pd(CG)₃, d(CG)₃ and pd(GC)₃ in 5 M NaCl. pd(CG)₃: at 0°C (——) and 25°C (———). *d(CG)₃: at 0°C (———). pd(GC)₃: at 0°C (— —). (* From fig.4 of [17].)

under such circumstances. Thus, comparison of the CD spectrum of pd(CG)$_3$ in 5 M NaCl at 0°C with those of Z-DNA (fig.2, d(CG)$_3$ in 5 M NaCl at 0°C) and B-DNA (fig.1, pd(CG)$_3$ in low salt at 0°C) suggests that the weak negative band at ~295 nm observed in pd(CG)$_3$ at 0°C in high salt can be attributed to the presence of a small fraction of Z-form in a B-Z mixture. On the other hand, pd(GC)$_3$ remains in the B-form in 5 M NaCl even at 0°C (fig.2). Therefore, it is clear that the presence of the 5′-phosphate in pd(CG)$_3$ inhibits the salt-induced B to Z transition.

To elucidate how the B-form is stabilized more than the Z-form in the presence of salt, we measured the UV-difference spectra between a low and a high temperature of both pd(CG)$_3$ and pd(GC)$_3$ in 1 and 5 M NaCl. The data are presented in fig.3a and b. It is evident from the UV-melting studies that both oligomers exist completely in the ordered structure below 15°C and in the denatured form by 70°C. In the case of pd(GC)$_3$, the UV-difference spectra recorded be-tween 70 and 10°C in 1 and 5 M NaCl are prac-tically identical. This indicates that there is no ef-fect of 5 M NaCl on the nature of stacking and conformation of the oligomer. Denaturation of the B-form gives only a positive UV-difference spec-trum with a maximum at ~277 nm. In contrast, pd(CG)$_3$ exhibits a small negative trough at ~295 nm and a strong positive band at ~274 nm in the UV-difference spectra (70–15°C) in 5 M NaCl, whereas d(CG)$_3$ exhibits a strong negative band around 295 nm observed for the denatura-tion of the Z-form. This reconfirms our earlier conclusions drawn from the CD studies that ad-dition of 5 M salt fails to induce complete Z-conformation in pd(CG)$_3$ as compared to d(CG)$_3$.

In 1 M NaCl both pd(CG)$_3$ and pd(GC)$_3$ exhibit the characteristic B-form CD spectra. The UV-melting profiles of pd(CG)$_3$ and pd(GC)$_3$ in 1 M NaCl show that both sequences undergo cooperative melting and have T_m values of 44.5 ± 0.5 and 48 ± 0.5°C, respectively (not shown). It is of interest that pd(GC)$_3$ is more stable in the B-
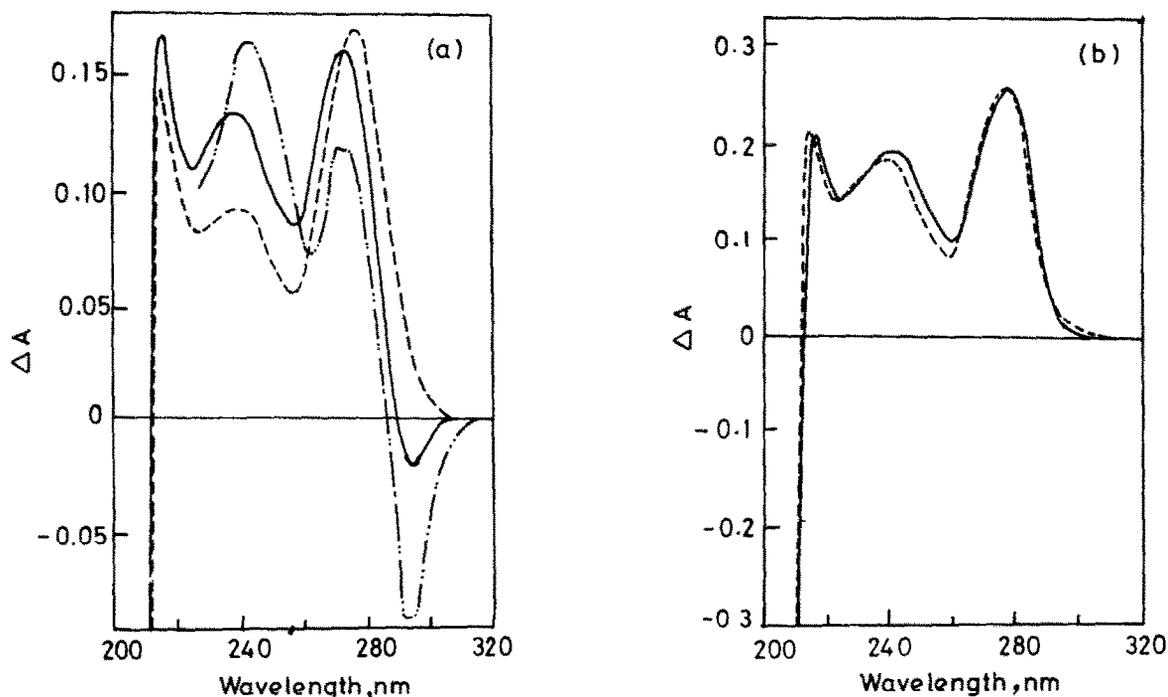

Fig.3. Difference-UV spectra of pd(CG)$_3$, d(CG)$_3$ and pd(GC)$_3$. (a) pd(CG)$_3$: (---) in 1 M NaCl for (70 – 10)°C, (——) in 5 M NaCl for (70 – 15)°C; *d(CG)$_3$: (—··—) in 5 M NaCl for (70 – 0)°C. (b) pd(GC)$_3$: (---) in 1 M NaCl for (70 – 10)°C, (——) in 5 M NaCl for (70 – 15)°C. (* Based on fig.1 of [17].)

form compared to pd(CG)₃. A similar observation has been reported for the same oligomers without the 5'-phosphate [19].

4. DISCUSSION

Comparison of the UV and CD spectra of pd(CG)₃ and pd(GC)₃ in low and high salt solutions at different temperatures shows that both oligomers exhibit an ordered conformation at low temperature. The spectral differences between pd(CG)₃ and d(CG)₃ clearly suggest that at room temperature the presence of a 5'-phosphate inhibits the salt-induced B ⟶ Z transition in pd(CG)₃. As d(GC)₃ does not undergo the salt-induced B ⟶ Z transition [19,20], the inhibitory effect of the 5'-phosphate group, if any, is redundant in pd(GC)₃.

Molecular model building studies of pd(CG)₃ were carried out to ascertain whether it was possible for stabilization of its B-conformation to occur specifically through extra H-bond formation involving the terminal 5'-phosphate. This was indeed found to be the case for formation of an intra-strand H-bond involving the hydroxyl of the monoprotonated phosphate group (as would be the case under the experimental conditions) and the pendant oxygen atom of the neighbouring phosphate group. Although such an intra-strand inter-phosphate H-bond can also be accommodated in the Z-form it is reasonable to assume that the formation of such an additional H-bond in the B-form will enhance the energy requirement for the B ⟶ Z transition in pd(CG)₃. The altered charge density of the molecule due to the 5'-phosphate group is quite likely to affect the stacking overlap of the bases, thereby changing the relative energy state of the B-form with respect to the Z-form. In fact, the presence of H-bonding involving the 5'-phosphate group in both forms cannot shift the B ⇌ Z equilibrium unless its energy is higher in one of these states. However, in the absence of detailed energy calculations, the model building per se cannot provide an unambiguous explanation of the observed 5'-phosphate effect.

As shown in fig.2, at low temperature this inhibitory effect is slightly less marked due to a possible shift in the B ⟶ Z equilibrium in favour of the Z-form. A phenomenon such as the shift in equilibrium towards the Z-form at lower temperature has in fact been observed in poly(dG-dC) under high salt conditions [22].

Recently, it has been shown that $n \geqslant 3$ and $n \geqslant 6$ are necessary for the d(CG)ₙ and d(GC)ₙ sequences, respectively to adopt the Z-conformation in high salt solution [19]. It has also been shown that the presence of a 5'-phosphate has no effect on the B ⟶ Z transition in d(GC)ₙ sequences [19]. Our studies do not contradict these results. It should be pointed out that in long sequences like pd(GC)ₙ ($n \geq 6$) the effect of 5'-phosphate on the B ⟶ Z transition is not expected to be significant, since the nucleation for the B ⟶ Z transition can take place at a point beyond the influence of the phosphate group, i.e. the effect of stabilization of the B-form is substantially reduced with increasing n. For exactly the same reasons, the long sequence of pd(CG)ₙ ($n \gg 3$) is unlikely to show any end phosphate effect. Interestingly, the higher stability of the d(GC)₃ sequence over that of d(CG)₃ in the B-form remains unaltered even when there is a free 5'-phosphate group on the oligomers. This difference may primarily arise due to a difference in stacking energy of the 5'-GpC-3' and 5'-CpG-3' sequence in the oligonucleotide chain in the B-conformation. Therefore, these results clearly indicate that critical charge neutralization and specific polarity of sequence can affect structural transitions in DNA.

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