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Conformational Effects on DNA Polymers Due to Physiological Concentrations of Divalent Metal Ions

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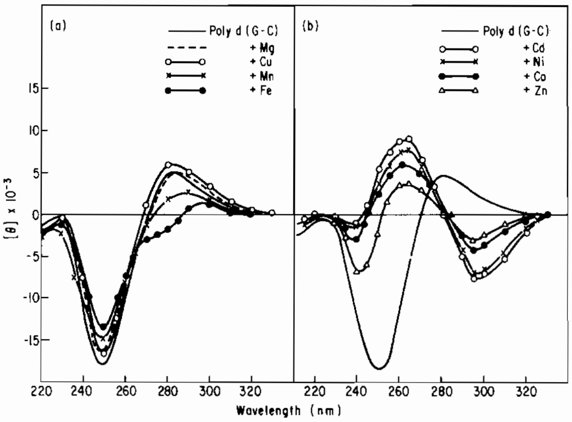

Fig. 1. CD spectra of polyd(G-C) in 0.02 *M* NaCl in the presence of various divalent metal ions. The 'r' values are 15 in Fig. 1a and 5 in Fig. 1b.

Since the discovery of left-handed helical conformations in synthetic DNA polymers with alternating G-C sequences [1, 2] the possibility of inducing such conformational transitions in other DNA polymers has received much attention. G-C polymers which contain modified guanine or cytosine residues have been shown to undergo the B to Z transition more readily than the polymer with unmodified bases [3, 4]. Another polymer with the alternating sequence, poly(dA-dC)·(dG-dT) also shows a limited ability to undergo such transitions at very high salt or ethanolic solutions [5, 6]. In our past studies, we have shown that the B-Z transition in poly d(G-C) can be brought about by physiological concentrations of certain divalent metal ions [7] without the aid of any other perturbing agents. We report here the results obtained by extending such studies to two other DNA polymers, poly(dG-m^5 dC) and poly(dA-dC)·(dG-dT) in the presence of various divalent metal ions at physiological concentrations in the absence of other perturbing agents.

Experimental

The synthetic DNA polymers polyd(G-C), poly(dG-m^{5}dC) and poly(dA-dC)·(dG-dT) were purchased from P. L. Biochemicals and Boeringer-Mannheim. All the three polymers were homogeneous in CsCl density gradient with an $S_{20,w}$ value ranging from 7.5 to 10. Stock solutions of the polymer were prepared in 0.2 *M* NaCl solutions made from double distilled, deionized water. The working polymer solutions which contained an average polymer concentration of 2.0×10^{-4} *M* nucleotide phosphorus, were prepared by appropriate dilutions of the stock solution with the deionized water to give NaCl concentration of 0.02 *M*. The metal ion to nucleotide phosphorus ratio in these studies is designated as the 'r' value. All the circular dichroic spectra were obtained in a Jasco 10 Spectropolarimeter with a temperature controlled cylindrical jacketted cell.

Results and Discussion

Figure 1 represents the CD spectra of polyd(G-C) in the presence of various divalent metal ions at an 'r' value of 5. The metal ions Cd(II), Ni(II), Zn(II) and Co(II) (Fig. 1b) bring about an inversion of the CD spectrum of polyd(G-C), presumably indicating the transition to the left-handed Z conformation. On the other hand, metal ions such as Cu(II), Mg(II), Mn(II) and Fe(II) (Fig. 1a) fail to bring about the inversion of the CD spectrum even at higher metal ion concentrations. The first set of metal ions which are known to be carcinogenic [8–10], possibly bring about the transition due to their selective affinity to bind to the N-7 position of guanine residues, which eventually leads to the rotation of the guanine residues to the 'syn' conformation which favors the Z conformation [1]. These results are discussed in much further detail in ref. 7.

Figure 2 shows the effects of these various divalent metal ions on the methylated polymer poly(dG-m^5 dc). The metal ions Cd(II), Ni(II) and Zn(II) bring about the inversion of the CD spectrum of this polymer even at lower metal ion to nucleotide phosphorus ratios. Manganese ions also initiate the inversion at these concentrations. The methylation of the 5 position of cytosine enhances the conformational flexibility of this polymer as compared to polyd(G-C). Behe and Felsenfeld [3] have shown that the B to Z

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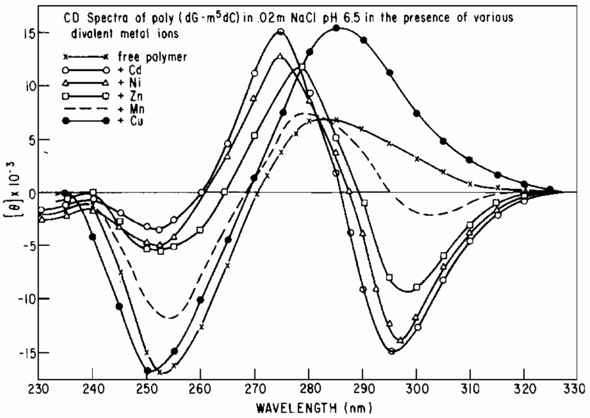

Fig. 2. CD spectra of poly(dG-m^{5}dC) in 0.02 *M* NaCl in the presence of various divalent metal ions. The 'r' values are 5 for Cd(II), Ni(II) and Zn(II) and 15 for Mn(II) and Cu(II).

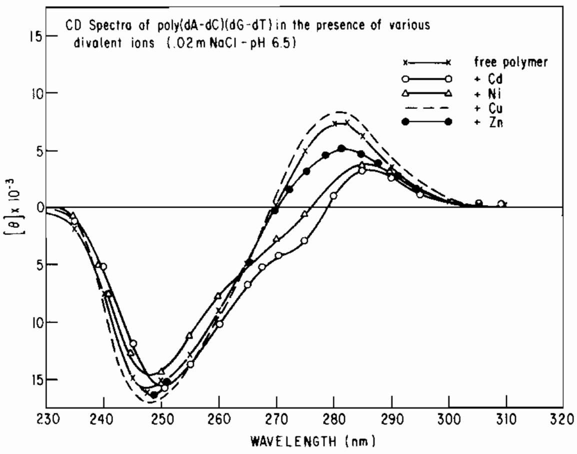

Fig. 3. CD spectra of poly(dA-dC)(dG–dt) in 0.02 *M* NaCl in the presence of divalent metal ions. The 'r' value is 15 in all the cases.

transition of this polymer can be brought about at NaCl concentrations of 0.7 *M* as against the 2.5 *M* NaCl required for the unmethylated polymer. We have shown in earlier studies that this polymer has even more flexibility at very low salt concentrations [11] and exhibits a second B to Z transition at NaCl concentrations of below 10 m*M*. The ease of transition of this polymer may well be due to the fact that the hydrophobic methyl group is more shielded from the solvent in the Z form but is more exposed to the solvent in the conventional B form [12]. The addition of divalent metal ions which bind to the N-7 of guanines and cause them to rotate to the 'syn' conformation, increases the rate of transition to the Z form further. In the unmethylated polymer, this factor seems to be the major driving force for the transition whereas in the methylated polymer, there are two driving forces involved, thus explaining the ease with which the methylated polymer undergoes the transition to the left handed conformation on binding with specific divalent metal ions.

On the other hand, the polymer poly(dA-dC)·(dG-dT) does not show the inversion of the CD spectrum even at metal ion to nucleotide phosphorus ratios of 15. However Cd(II), Ni(II) and Zn(II) ions reduce the positive ellipticity of the CD spectrum at these concentrations. This type of CD spectrum is indicative of an intermediate conformation or a modified B form [5] encountered during the B to Z transition. At higher metal ion concentrations it may be possible to obtain the transition to Z form. Attempts to increase the metal ion concentrations to very high values resulted in turbidity of the polymer solution resulting in erratic spectra.

In all the three polymers, the effects of Cu(II) ions stand out significantly. In polyd(G-C), concentrations of Cu(II) ions as high as 15 times that of the polymer, does not cause any appreciable trend towards the B to Z transition and in fact a slight increase in the positive ellipticity is noted. In the methylated polymer, the effect of Cu(II) is even more significant in that a very large increase in the positive ellipticity is observed. Such increase is also noted in poly(dA-dC)·(dG-dT) though to a lesser extent. These spectra of the polymer–Cu(II) complexes are indicative of an A like helical conformation which is usually characterized by a nonconservative spectrum with a high positive ellipticity [13]. Copper which is a noncarcinogenic metal, also has a binding affinity to N-7 and O-6 of guanine residues and forms strong complexes as shown by X-ray crystallographic studies [14] but analysis of such Cu(II)–Guanine complexes by Sletten and Li [15] show that binding of Cu(II) ions does not rotate the guanine residues to the 'syn' conformation and that the binding of Cu(II) actually is able to hold the guanine–cytosine pair in the conventional Watson–Crick base pairing. Thus Cu(II) enhances the stability of the B form rather than the Z form. In the methylated polymer, the effect of Cu(II) ions significantly tips the B to Z transition equilibrium to the B form, thus overcoming the effect of methylation of cytosine in favoring the Z form. The increase in positive ellipticity indicative of an 'A' conformation can be related to the changes in the tilt of the base pairs due to the binding of Cu(II) to guanine residues.

A comparison of these results on the three different polymers indicates that the actual rotational conformation of the bases in the helix may be a significant factor in influencing the rate of B to Z transition. Since these results have been obtained at physiological concentrations of the metal ions, the dehydrating effects due to high salt concentrations or any other perturbing agents are absent and the effects are exclusively due to the complexing of the metal ions with the polymers. The biological relevance of the Z conformation in the G-C rich regions of chromosomal DNA [26] have been explored for such transitions *in vitro,* questions as to whether the Z DNA can exist *in vivo* under physiological conditions have been posed. From our present results, we can predict that the chemical agents such as the metal carcinogens can bring about the transition to the Z form very easily under physiological conditions in susceptible segments of chromosomal DNA.

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