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Differential promotion and suppression of Z→B transitions in poly[d(G-C)] by histone subclasses, polyamino acids and polyamines

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The right-handed (B) conformation of poly[d(G-C)] in 7.5 mM sodium cacodylate and 25% ethylene glycol can be readily converted to the left-handed (Z) conformation by the addition of 250 μM $MnCl_2$ and this transition can be reversed by chelation of the Mn ions with EDTA or by addition of NaCl. This ability to obtain such reversible transitions in solvent and solute conditions which allow DNA-protein interactions and their assessment by c.d. permitted an analysis of the effect of purified histones, polyamino acids, protamine and polyamines on these transitions. Individual core histones H3, H4, H2a and H2b or protamine stabilised the Mn-induced Z form and prevented the transition to B DNA normally observed after chelation with EDTA or on dialysis to physiological salt concentrations. A similar suppression of Z→B transition was also achieved with poly-L-arginine (but not with poly-L-lysine). In contrast, histones H1 and H5 promoted the Z→B transition. Polyamines (spermine and spermidine) converted the B form to another right-handed (A) form which transformed to the Z form after the addition of EDTA and this Z form was restored to the B conformation on the addition of NaCl. These results suggest that sequence-dependent variations in the conformation of natural DNA may be modulated by interaction with histones and other basic cellular components and may provide a conformational basis for nucleosome formation and possibly for the control of gene expression.

Key words: Z DNA/polyamines/protamine/histones/circular dichroism

Introduction

There is now good evidence that alternating (dG-dC) double-stranded sequences can, under the appropriate conditions, exist as a left-handed helix (for reviews, see Cantor, 1981; Dickerson *et al.*, 1982). The transition from the standard right-handed B-DNA to the left-handed conformation can be induced in a variety of ways, the most common being high concentration of NaCl (>3.5 M) or ethanol (>50%) (Pohl and Jovin, 1972; Pohl, 1976) – conditions which are non-physiological. The conversion, however, can be induced in physiological conditions by the introduction of supercoiling (Singleton *et al.*, 1982). The model for the left-handed DNA from X-ray diffraction data (Wang *et al.*, 1979; Drew *et al.*, 1978), differs from the right-handed B form in a number of ways, with the sugar-phosphate backbone adopting an irregular zig-zag conformation, hence the term 'Z DNA'. In addition, the guanine bases are rotated 180° about the glycosidic bond from the *anti* to the *syn* conformation so that the 7 and 8 positions of the guanine are on the outer surface and there is only one groove present. Methylation of dC residues in poly[d(G-C)] strongly facilitates the salt conversion from B to Z form, suggesting that one role of DNA methylation may be to regulate gene expression by inducing changes in DNA conformation (Behe and Felsenfeld, 1981). Nevertheless conditions have not yet been obtained which allow the conversion of unmodified (dG-dC) sequences to the Z form to be retained in this conformation using normal cellular components in a physiologically favourable environment. There has been one report (Klevan and Schumaker, 1982) that addition of poly-L-arginine (but not poly-L-lysine) to poly[d(G-C)] in high salt will allow the retention of the Z configuration when the salt concentration is reduced to physiological levels. In an attempt to discover conditions which are more biologically compatible than the previously reported high salt or ethanol, some workers (van de Sande *et al.*, 1982; Zacharias *et al.*, 1982) have explored the use of divalent ions acting synergistically with dehydrating agents such as ethanol and ethylene glycol.

We have utilised one of these methods, (*viz.* the ability of micromolar amounts of $MnCl_2$ in the presence of 25% ethylene glycol to convert poly [d(G-C)] to the Z form), to study, by c.d., the effect of adding basic proteins on the ease of interconversion of the polynucleotide conformations. We have discovered that some basic proteins appear to bind tightly to either the B or Z forms preventing the interconversion whereas others stabilize the B form selectively. The core histones H3, H4, H2a, and H2b, bind to either Z or B forms whereas histones H1 and H5 actively favour the B conformation. Moreover, after binding the core histones to poly[d(G-C)] in the Z form, the polymer retains the Z conformation even under physiological salt conditions.

Results

Optimum conditions for interconversion of B and Z forms

The transition from the B to the Z form was monitored by analysing the characteristic changes in the c.d. of poly[d(G-C)]. The Z conformation shows decreased amplitude and a sign inversion in the 295 nm region and a decrease in the negative c.d. in the 250 nm region. We examined a number of conditions which utilised the divalent transition metal ions in combination with dehydrating agents such as trifluorethanol, ethanol and ethylene glycol and finally selected 7.5 mM sodium cacodylate pH 7.5, 250 μM $MnCl_2$ and 25% ethylene glycol (Zacharias *et al.*, 1982) as the most satisfactory (Figure 1A). Thus, relatively low ionic strength and solvent concentrations were employed, no heating was necessary and apparently no fast sedimenting Z forms (Z*) (van de Sande and Jovin, 1982) were generated. The intensities of the spectra appear to be somewhat dependent on the exact solvent conditions but the basic conformational forms (B and Z) are readily distinguishable. In agreement with a previous report, we noted that the conversion from B to Z on adding $MnCl_2$ was time dependent and we routinely allowed at least 15 min for equilibration. It was also apparent that the reverse change from Z to B could be rapidly accomplished by adding EDTA to 500 μM (Figure 1B), or by adding NaCl to

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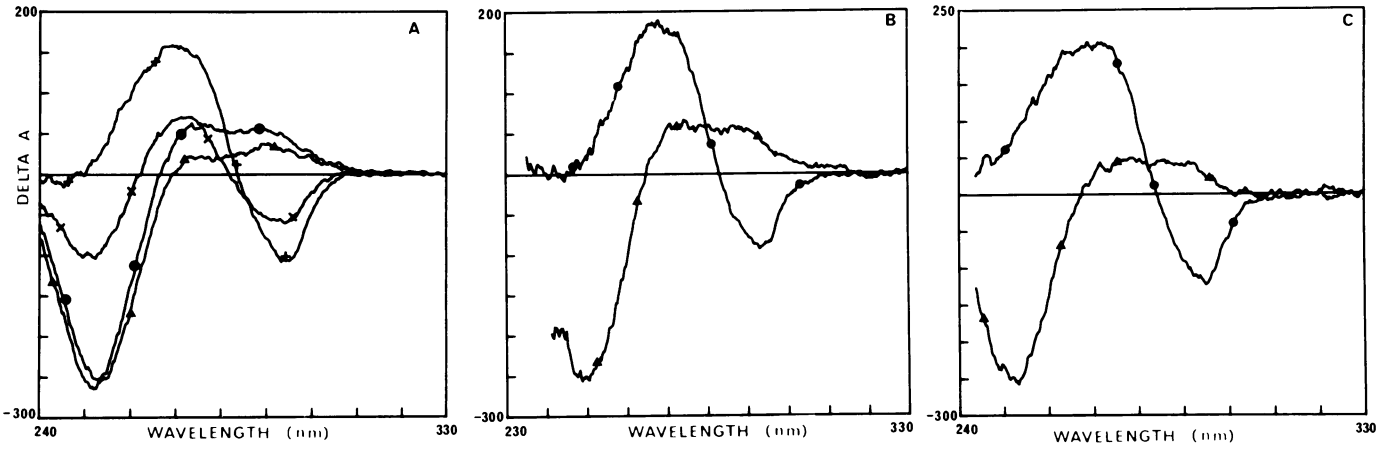

Fig. 1. Interconversion of Z and B forms of poly[d(G-C)]. **(A)** C.d. spectra of poly[d(G-C)] in 7.5 mM sodium cacodylate 25% ethylene glycol ——●——●——, with $MnCl_2$ added to 50 μM ——▲——▲——, with $MnCl_2$ added to 150 μM ——x——x——, with $MnCl_2$ added to 300 μM ——+——+——. **(B)** C.d. spectra of poly[d(G-C)] in 7.5 mM sodium cacodylate, 25% ethylene glycol, 250 μM $MnCl_2$ (buffer A) ——●——●——, after addition of EDTA to 500 μM ——▲——▲——. **(C)** C.d. spectra of poly[d(G-C)] in buffer A ——●——●——, after addition of NaCl to 150 mM ——▲——▲——.

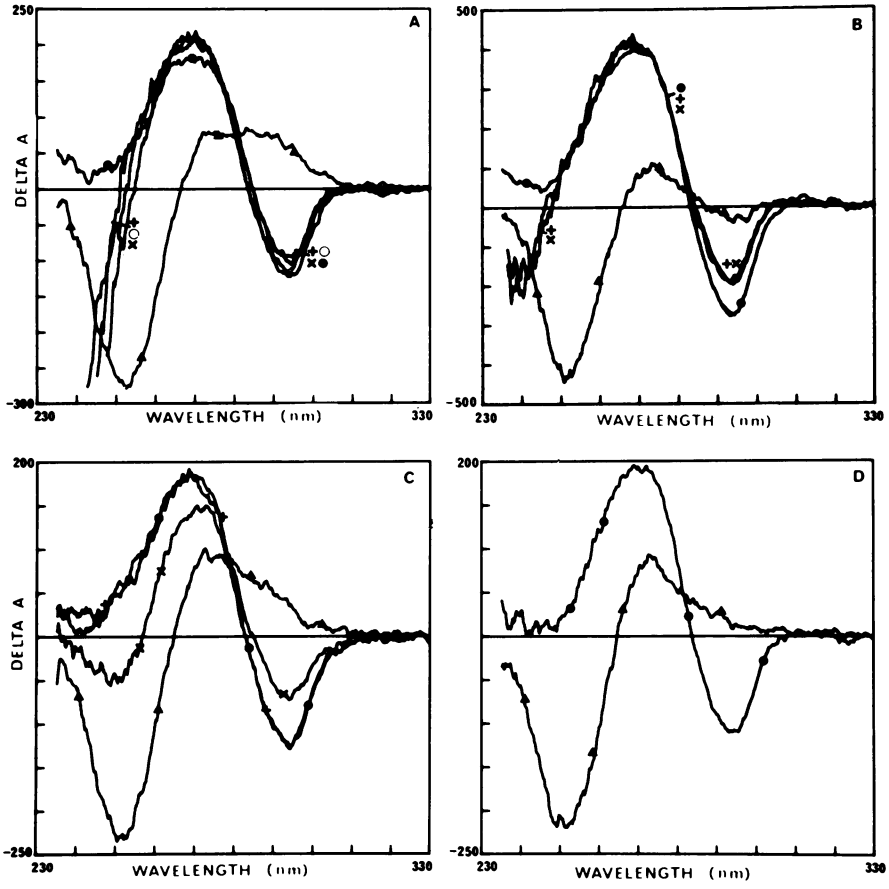

Fig. 2. Effect of adding poly-L-arginine and poly-L-lysine on Z→B conversions. **(A)** C.d. spectra of poly[d(G-C)] in buffer A ——●——●——, after addition of EDTA to 750 μM ——▲——▲——, after addition of 2 μg of poly-L-arginine, ——+——+——, after addition of 2 μg of poly-L-arginine followed by EDTA to 750 μM ——○——○——, after addition of 2 μg poly-L-arginine followed by EDTA to 1.5 mM ——x——x——. **(B)** C.d. spectra of poly[d(G-C)] in buffer A ——●——●——, after dilution with 7.5 mM sodium cacodylate (1:2) ——▲——▲——, after addition of 2 μg poly-L-arginine ——+——+——, after addition of 2 μg poly-L-arginine followed by dilution with 7.5 mM sodium cacodylate (1:2) ——x——x——. (Spectra are adjusted for dilution where appropriate). **(C)** C.d. spectra of poly[d(G-C)] in buffer A, after addition of 200 ng of poly-L-arginine followed by EDTA to 500 μM ——▲——▲——, after addition of 1 μg of poly-L-arginine followed by EDTA to 500 μM ——x——x——, after addition of 2 μg poly-L-arginine followed by EDTA to 500 μM ——+——+——. **(D)** C.d. spectra of poly[d(G-C)] in buffer A after addition of 2 μg poly-L-lysine ——●——●——, after addition of 2 μg poly-L-lysine followed by EDTA to 500 μM ——▲——▲——.

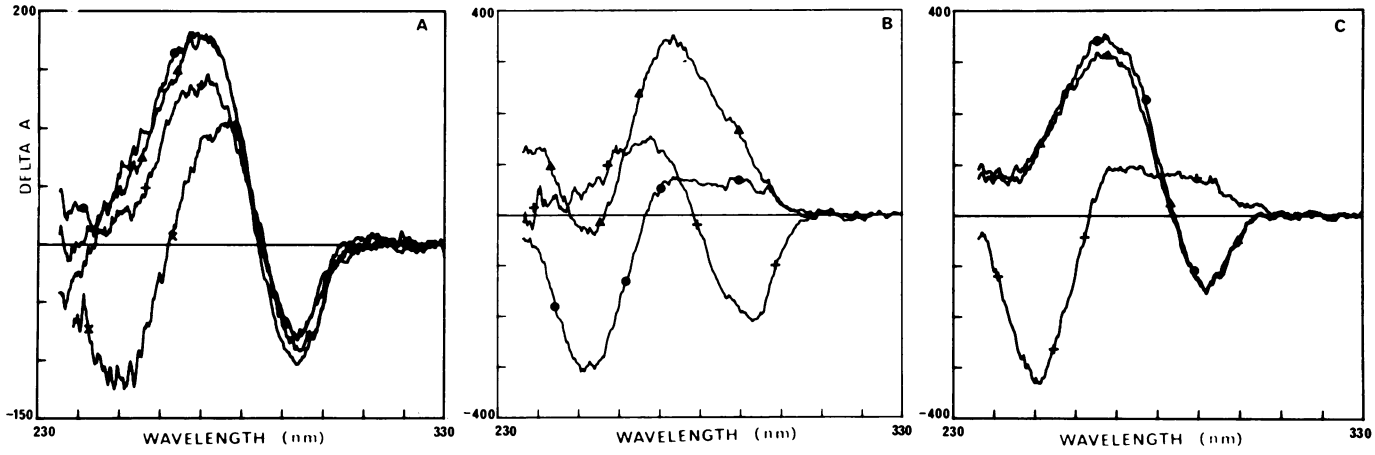

Fig. 3. Effect of adding protamine and polyamines on the Z→B conversion. (**A**) C.d. spectra of poly[d(G-C)] in buffer A ——●——●——, after addition of 50 μg protamine ——▲——▲——, after addition of 50 μg protamine followed by EDTA to 500 μM ——+——+——, after addition of 50 μg protamine followed by NaCl to 150 mM ——x——x——. (**B**) C.d. spectra of poly[d(G-C)] in 7.5 mM sodium cadocylate pH 7.5 in 25% ethylene glycol ——●——●——, after addition of 50 μg spermine ——▲——▲——, followed by further addition of EDTA to 500 μM ——+——+——. (**C**) C.d. spectra of poly[d(G-C)] in buffer A ——●——●——, after addition of 50 μg spermidine ——▲——▲——, then followed by NaCl to 150 mM ——+——+——.

Table I. Sources of basic proteins and polyamines

Reagent	Source	Comments
Poly-L-arginine hydrochloride	Sigma Chemical Co.	Mol. wt. 44 000
Poly-L-lysine hydrobromide	Sigma Chemical Co.	Mol. wt. 66 000
Spermine tetrahydrochloride	Sigma Chemical Co.	–
Spermidine trihydrochloride	Sigma Chemical Co.	–
Protamine sulphate (salmon)	Sigma Chemical Co.	–
Histone H1 (pig)	Institute of Cancer Research (ICR)	Doublet polypeptide band in SDS-PAGE
Histone H1 (calf)	Boehringer-Mannheim (BM)	Doublet polypeptide band in SDS-PAGE
Histone H2A (pig)	ICR	Single polypeptide band in SDS-PAGE
Histone H2B (calf)	ICR	Single polypeptide band in SDS-PAGE
Histone H2B (calf)	BM	Single polypeptide band in SDS-PAGE
Histone H3 (pig)	ICR	Single polypeptide band in SDS-PAGE
Histone H4 (pig)	ICR	Single polypeptide band in SDS-PAGE
Histone H4 (rabbit)	BM	Single polypeptide band in SDS-PAGE
Histone H5 (chick)	ICR	Single polypeptide band in SDS-PAGE

150 mM (Figure 1C). Dilution of the solution to $[Mn^{2+}] < 100$ μM (see Figure 2B) also facilitated the reversal.

Effect of adding polyarginine and polylysine

Studies by Klevan and Schumaker (1982) had shown that, on addition of poly-L-arginine to poly[d(G-C)] in high salt (>3.5 M NaCl), the Z conformation was retained on dilution to 170 mM NaCl. Utilising the $MnCl_2$/ethylene glycol system, we find that addition of poly-L-arginine to the poly[d(G-C)] in the Z form prevented the reverse transformation to the B form either on addition of EDTA (Figure 2A) or on dilution of $[Mn^{2+}]$ to 80 μM (Figure 2B). Titration of ~15 μg of poly[d(G-C)] revealed that 2 μg of poly-L-arginine was sufficient to prevent the Z to B transformation (Figure 2C). Similar experiments carried out with poly-L-lysine indicated that this polymer did not prevent the Z→B conversion on addition of 500 μM EDTA (Figure 2d). Adding either poly-L-arginine or poly-L-lysine to poly[d(G-C)] in the B form prevented the conversion to the Z form on addition of $MnCl_2$ (data not shown), indicating that poly-L-lysine is capable of interacting with poly[d(G-C)] in the B form.

Effect of adding protamine and polyamines

Similar experiments were carried out using protamine, spermine and spermidine and Figure 3A shows that, on adding protamine to the Z form of poly[d(G-C)], the left-handed conformation, as in the case of poly-L-aginine, was retained in the presence of EDTA. Retention of the Z form by protamine was also apparent with some perturbation on adding NaCl to 150 mM (Figure 3A). On addition of protamine to the B form of the polymer, no conversion to the Z form could be induced by $MnCl_2$ (data not shown).

With polyamines, different effects were noted. On adding either spermine or spermidine to poly[d(G-C)] in the B form in the presence of ethylene glycol, another right-handed form, the A form, was obtained. This form is characterised by a

positive c.d. at 270 nm (Minyat *et al.*, 1978). No conversion to the Z form was observed on addition of $MnCl_2$ although, surprisingly, the Z conformation was obtained with the subsequent addition of EDTA to 500 μM. Indeed, the left-handed conformation was produced on merely adding EDTA to the A form induced by the spermine (or spermidine) i.e., without $MnCl_2$ (Figure 3B). However, in either case, on addition of NaCl to 150 mM (data not shown), the Z form was transformed back to the B form – as was the A form. Similarly the Z form induced by $MnCl_2$ and retained on addition of spermine was converted back to the B form on addition of NaCl to 150 mM (Figure 3C).

Effect of adding histones

The histone samples (see Table I) were obtained from a number of sources and only those which were judged to be at least 95% pure, as characterised by the appropriate polypeptide electrophoretic mobility in SDS-polyacrylamide gel electrophoresis (PAGE), were utilised. They were added to either the Z or B form of poly[d(G-C)] in 7.5 mM sodium cacodylate, 25% ethylene glycol. Reference to Figure 4 shows that, with histones H2a, H2b, H3 or H4, the polymer essentially retained the Z conformation after addition of NaCl to 150 mM, although there were significant perturbations in some of the c.d. spectra (e.g., Figure 4B,D). Figure 4C and E also indicates that a similar retention of the left-handed conformation could be achieved by addition of EDTA. It should be noted that different sources of histones yielded similar results. Addition of 150 mM NaCl to ~15 μg of Z form poly[d(G-C)] complexed with different amounts of histone H3 or H4 showed that ~10–20 μg of either histone could maintain ~50% of the polymer in the left-handed form (data not shown). A further separate experiment also clearly indicated that the Z form of poly[d(G-C)] was substantially retained in the presence of either H3 or H4 histone after ethylene glycol and Mn^{2+} had been removed by dialysis against 500 volumes of 10 mM Tris/HCl pH 7.8, 150 mM NaCl (Figure 5). A similar investigation using histones H2a and H2b also showed that with these histones the polymer retained the Z conformation after dialysis (data not shown).

On analysing the behaviour of histones H1 and H5 in this system, further differences were observed. Addition of either of these two histones to the Z form of poly[d(G-C)] actively promoted the conversion to the B form even in the absence of EDTA or NaCl (Figure 6). It was also apparent that some aggregation (as indicated by light scattering) was occurring (particularly with H1) as the B form was produced. In agreement with the results for the other basic proteins, any of the

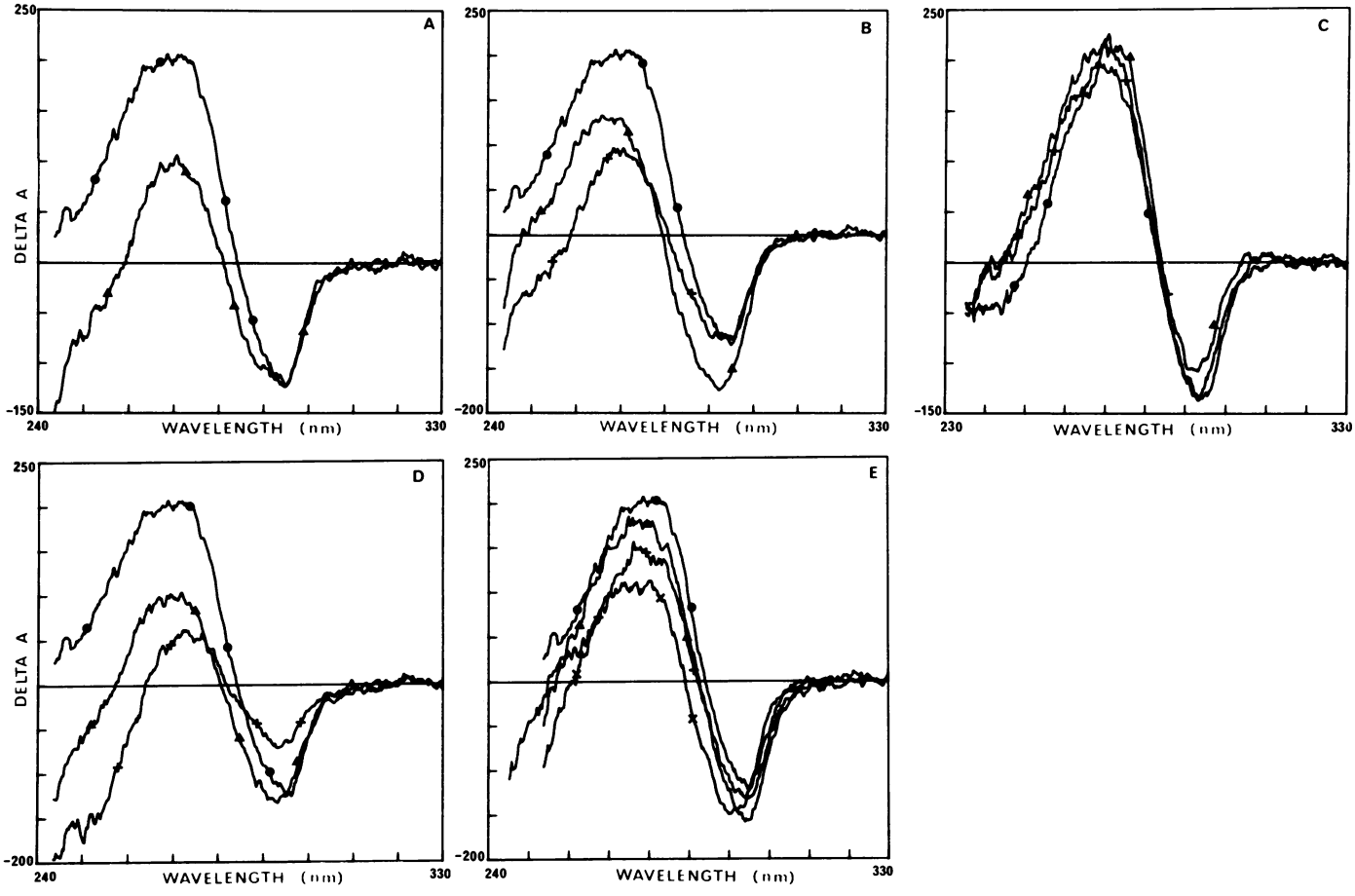

Fig. 4. Effect of adding core histones on the Z→B conversion. **(A)** C.d. spectra of poly[d(G-C)] in buffer A —●—●—, after addition of 20 μg of histone H2a followed by NaCl to 0.15 M —▲—▲—. **(B)** C.d. spectra of poly[d(G-C)] in buffer A —●—●—, after addition of 20 μg of histone H2b (ICR) —▲—▲—, then followed by addition of NaCl to 0.15 M —+—+—. **(C)** C.d. spectra of poly[d(G-C)] in buffer A —●—●—, after addition of 20 μg of histone H2b (BM) —▲—▲—, then followed by addition of EDTA to 500 μM —+—+—. **(D)** C.d spectra of poly[d(G-C)] in buffer A —●—●—, after addition of 20 μg of histone H3 —▲—▲—, then followed by addition of NaCl to 0.15 M —+—+—. **(E)** C.d. spectra of poly[d(G-C)] in buffer A —●—●—, after addition of 20 μg of histone H4 —▲—▲—, then followed by addition of NaCl to 0.15 M —+—+— or followed by addition of EDTA to 500 μM —x—x—.

histones were able to prevent the transformation of the B to the Z form by $MnCl_2$ in the presence of ethylene glycol (data not shown).

Effect of adding competing reagents to poly[d(G-C)]

To assess qualitatively the relative effectiveness of histones and polyamines in this system, the effect of adding H4 histone to poly[d(G-C)] in the presence of spermine was examined. It was evident that the A form could be stabilised by the presence of spermine, since the addition of H4 and $MnCl_2$ did not induce the Z conformation. However, the B form was also obtained on addition of NaCl either with or without the H4 histone (data not shown).

The stability to reversion by NaCl of Z form poly[d(G-C)] in the presence of H4 histone and increasing amounts of H1 histone was studied by measuring c.d. at 295 nm. The Z form of poly[d(G-C)] was complexed with the optimal amount of H4 histone (i.e., that amount just sufficient for the polymer to retain the Z form on addition of NaCl) and variable amounts of H1 histone added followed by NaCl to 150 mM. The results (not shown) indicate that the proportion of B obtained by adding NaCl was progressively enhanced by increasing amounts of H1 histone. Moreover, it was noted that there was very little evidence of aggregation as occurred when adding H1 histone alone to the Z form of the polymer.

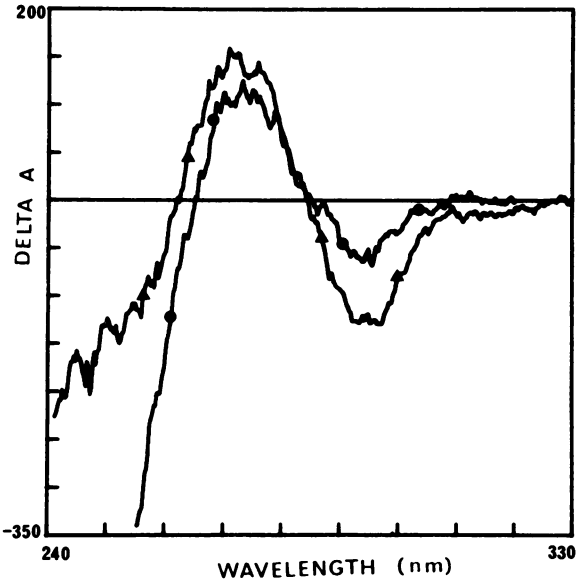

Fig. 5. Effect of adding H3 and H4 histones on the Z→B conversion and retention of Z structure under physiological salt conditions. C.d spectra of poly[d(G-C)] in buffer A after addition of 20 μg of histone H3 and addition of NaCl to 150 mM followed by dialysis against 150 mM NaCl, 10 mM Tris pH 7.8 ——●——●——; similarly with histone H4 ——▲——▲——.

Discussion

These experiments (summarised in Figure 7) have shown that interconversion of poly[d(G-C)] between the right-handed B form and the left-handed Z form can be readily achieved at low ionic strength in ethylene glycol using the opposing effects of $MnCl_2$ and EDTA (cf., van de Sande *et al.*, 1982; Zacharias *et al.*, 1982). The conversion of Z→B form can also be accomplished by addition of NaCl to 150 mM presumably by reducing the ability of Mn^{2+} ions to provide strong electrostatic shielding along the phosphate backbone of the Z form (Granot and Kearns 1982). There is also some evidence that Mn^{2+} can interact directly with the exposed N7 of guanine as well as with phosphate groups (Berger and Eichorn, 1971), thus suggesting that specific effects may be involved in addition to relatively non-specific electrostatic interactions.

Using appropriate conditions, we have shown that certain basic proteins, e.g., histones H3 and H4 and poly amino acids (poly-L-arginine), can interact with the poly[d(G-C)] to 'lock' the conformation in the Z form in a physiologically favourable environment. In contrast, poly-L-lysine and histones H1 and H5 very clearly preferred the B form. The polyamines behaved rather differently in that they appeared to favour the formation of the A form of the polymer. It had previously been noted by Minyat *et al.* (1978) that B form DNA can be converted to the A form in the presence of ethanol and either spermine or spermidine and it has been further suggested that the A form of poly[d(G-C)] can function as an intermediate in the interconversion of the B and Z forms (Ivanov and Minyat, 1981). Our observation of the conversion of the polyamine-induced A form to the Z form in the presence of EDTA was surprising and may reflect the modulation of the effect of the polyamine following interaction with the chelating agent.

The major finding of these studies is that in the presence of core histones H3, H4, H2a or H2b, poly[d(G-C)] can retain the Z conformation at physiological salt concentrations and

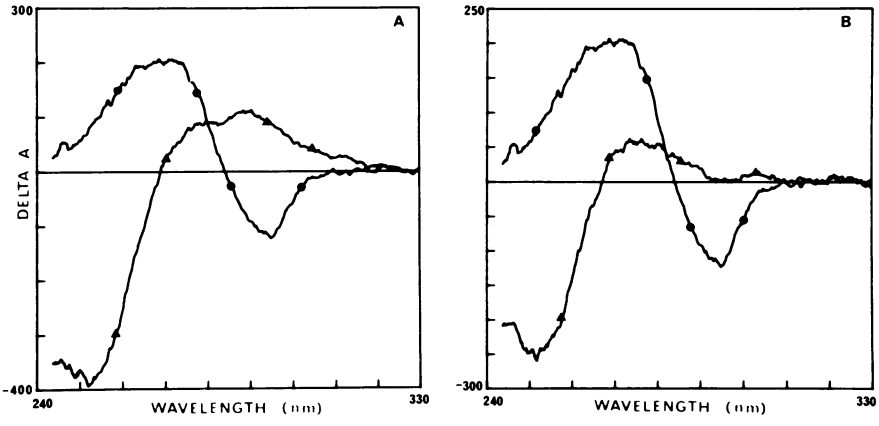

Fig. 6. Effect of adding H1 and H5 histones on the Z→B conversion. **(A)** C.d. spectra of poly[d(G-C)] in buffer A ——●——●——, and after addition of 20 μg of histone H1 ——▲——▲——. **(B)** C.d. spectra of poly[d(G-C)] in buffer A ——●——●—— and after addition of histone H5 ——▲——▲——.

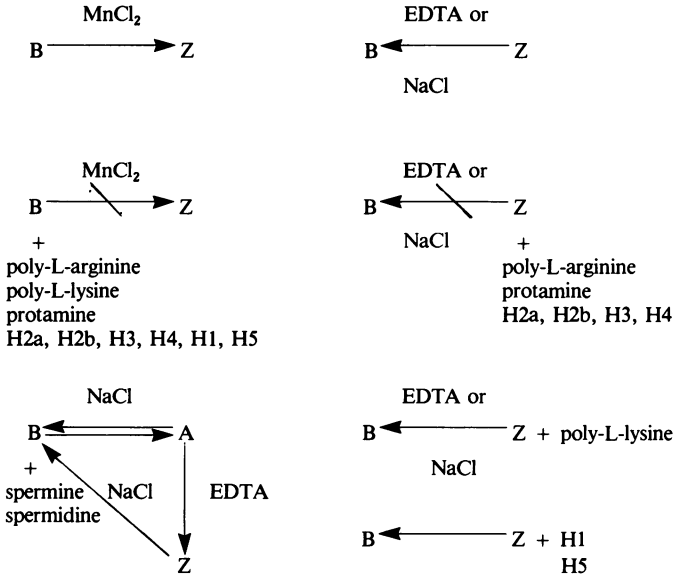

Fig. 7. Summary of Z⟷B interconversions

on removal of the dehydrating agent. In contrast, either histone H1 or H5 can induce the conversion from Z to B in the absence of either EDTA or salt. It is notable that the amount of histones required to demonstrate the effects were consistent with the ratios of DNA to histone found in isolated chromatin and, therefore, reflects a situation which is in this respect comparable to that found *in vivo*. It is also interesting that the stabilization of the Z form by the separate core histones can apparently be reversed by the addition of H1 histone and it may be that the histone octamer (see below) can form a somewhat more stable Z-binding structure.

These results, obtained with the synthetic alternating co-polymer, are clearly of interest with regard to the nature of the binding of histones to native DNA. Indeed, it is tempting to suggest that core histones may bind preferentially to such left-handed conformations in natural DNA and may thereby play a major role in the formation of nucleosomes. These structures are now accepted as being initially formed from the interaction of H3, H4 tetramers with DNA followed by histone octamer formation with histones H2a and H2b (for review, see Bradbury, 1982). The fact that H1 (and the related H5) histone appears to favour the B form and also seems to interact independently of the core histones, would be compatible with the suggestion that local changes (Z⟷B) in DNA conformation might be important in facilitating higher order packaging of the nucleosomes, and might also be related to the presence of DNase-hypersensitive sites in chromatin. It is interesting that the arginine-enriched proteins appear to interact more strongly with the Z form of the polymer and it may be that the guanidino side chain of arginines can hydrogen bond in a very specific manner with the guanine bases of the polymer when they are in the *syn* conformation (c.f., Seeman *et al.*, 1976).

This suggestion, however, is somewhat at variance with the findings of Nickol *et al.* (1982) who examined the ability of core histones to reconstitute nucleosomes on both poly[d(G-C)] and poly[d(G-m^{5}C)] using different conditions for interconversion of the Z and B forms from those described in this study. In the B form, the alternating co-polymer appeared to form nucleosomes, as determined by a number of criteria [cf., Simpson and Kunzler (1979)], but they were unable to detect nucleosomes by the same criteria using the methylated polymer when the Z form was induced in the presence of 0.2 M NaCl, 10 mM Tris pH 8.0 and 0.55 mM Co $(NH_3)_6^{3+}$, apparently producing instead a fast sedimenting aggregated complex. On the basis of these experiments, Nickol *et al.* suggested that the presence of the Z form prevented the formation of nucleosomes. In the light of these apparently conflicting observations it would be instructive to carry out similar experiments to those of Nickol *et al.* under the interconversion conditions described here and using the unmethylated form of the polymer in the Z form. It does seem possible that the presence of the methyl groups on the cytosines could effectively block the interaction of the histones with the appropriate bases particularly if they are in a different configuration from the B form. Studies were also made by Simpson and Shindo (1980) to assess the possible role of core histones in promoting a Z conformation in poly[d(G-C)]. By reconstituting directly onto the polymer these workers, however, in agreement with the results described here for the isolated core histones, were unable to obtain any indication of the B→Z transition. More recent results by Miller *et al.* (1982) have shown that nucleosome formation on a Z DNA template may not be incompatible.

Other studies have indicated the presence of Z DNA structures in polytene chromosomes and plasmids (Nordheim *et al.*, 1981, 1982; Arndt-Jovin *et al.*, 1983; Klysik *et al.*, 1981). In the latter case, the Z conformation appeared to be associated with a very limited stretch of alternating purine-pyrimidine sequences (14 bases with one residue out of phase). Our own computer searches on published DNA sequences for alternating purine-pyrimidine bases have revealed that stretches of eight bases or more occur at frequencies along the DNA not inconsistent with a relationship to nucleosome formation. Thus, the sequence of ~12 000 bases at the left hand end of adenovirus 2 (Gingeras *et al.*, 1982) DNA contains at least 24 different such stretches, i.e., ~1 per 500 bases. It was also observed that groups of such alternating sequences with six or more bases occurred fairly frequently and it may be that such clusters could be of some significance.

It is pertinent to note that the results described in this study are consistent with the possible existence of a family of related left-handed Z conformations and these may be differentially stabilized by the histones and protamine, although it cannot be ruled out that some of the observed variability in the c.d. spectrum of the Z form complexes could be the result of purely spectroscopic effects, e.g., transition cooperativity, effect of length of polymer segments on optical properties, etc. (cf. Zacharias *et al.*, 1982; van de Sande *et al.*, 1982). It should also be borne in mind that c.d. spectra do not unambiguously prove that a polymer exists in a given form (Tomasz *et al.*, 1983). Since DNA molecules *in vitro* do not necessarily have a uniform conformation represented by the classical B form but rather have regions of sequence-dependent variations (Klug *et al.*, 1979; Lomanossof *et al.*, 1981; Dickerson and Drew, 1981) it seems plausible to speculate that some of these regions may exhibit a distinct and differential facility for conversion to the left-handed forms. Of further relevance is the suggestion that some degree of 'phasing' of nucleosomes in chromatin can occur, implying a degree of specificity in the DNA-histone interaction (for reviews, see Kornberg, 1981; Zachau and Igo Kemenes, 1981). Thus, although the studies have been carried out here

using the synthetic poly[d(G-C)] the results are in keeping with the suggestion that local perturbations in the handedness of the natural DNA strand may be important in providing 'nucleation' sites for the initial H3-H4 interaction for nucleosome formation. This could occur particularly if the histone interaction is coincident with DNA replication, and methylation or other modifications could allow the histones to interact on local and even temporary changes in conformation. The ubiquitous nature of polyamines (and protamines), as well as the histones, raises the question of how far these basic components stabilise and accentuate local changes in conformation thereby allowing the specific binding of other proteins, possibly leading in turn to modulation and regulation of gene expression.

Materials and methods

Reagents

Poly[d(G-C)] was obtained from P.L. Biochemicals and had an $S20_W$ of ~10 and an apparent mol. wt. on analysis by agarose gel electrophoresis of $\sim 5-15 \times 10^5$ daltons. The sources of the basic proteins and polyamines are given in Table I. The histones kindly donated by E.W. Johns and R. Nicholas of the Institute of Cancer Research had been initially extracted using the techniques outlined by Johns (1977) and histones H2a, H2b, and H4 further purified using molecular sieving methods (van Holt and Brandt, 1977) and histones H1 and H5 using carboxymethylcellulose chromatography (Goodwin and Johns, 1977). The histones were analysed by standard techniques of SDS-PAGE (Russell and Blair, 1977) and quantitated by their u.v. absorption at 280 nm in 0.1 N HCl (Johns, 1971). In some experiments, histones H1 and H5 were dissolved in demineralised water and dialysed against 7.5 mM sodium cacodylate buffer to ensure that there was no residual salt in the preparations.

C.d.

Poly[d(G-C)] was dissolved in 7.5 mM sodium cacodylate pH 7.8 at a concentration of 1 mg/ml and diluted into 1 ml of the appropriate buffer to give an O.D. at 260 nm of 0.30. Normally c.d. measurements were made in 7.5 mM sodium cacodylate pH 7.8 containing 25% ethylene glycol ('Analar', B.D.H. Poole, UK) and spectra were recorded at 22°C in a Jasco J41C spectropolarimeter equipped with a model J-DPY Data Processor. Fused silica cells of 10 mm pathlength were used to record spectra from 340 to 230 nm, at a sensitivity of 1 m-degree/cm, with an instrumental time constant of 4 s. The spectra shown are presented in terms of differential absorbance, $10^6 \times \Delta A$. All the c.d. spectra were recorded within 30 min of completing a given experiment and appeared to reflect equilibrium conditions since there were no significant changes in spectra after longer periods. (This was checked particularly after dialysis of histone-polymer complexes.).

U.v. absorption

These spectra were recorded in a Beckman DU-7 spectrophotometer.

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