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Interactions of Poly(N^{ϵ},N^{ϵ},N^{ϵ}-trimethyllysine) and Poly(lysine) with Polynucleotides: Circular Dichroism and A-T Sequence Selectivity†

Edward N. Granados and Jake Bello*

ABSTRACT: Complexes of $(Lys)_n$ and $[Lys(Me_3)]_n$ with natural and synthetic DNAs have been studied by CD as a function of ionic strength. In dilute EDTA, $(Lys)_n$ and $[Lys(Me_3)]_n$ produce the same distortions to the CD spectrum of calf thymus DNA at r (peptide residue/nucleotide residue) values < 0.6. At higher r values, the distortions are somewhat different. $[Lys(Me_3)]_n$ alters the conformation of some polynucleotides differently from $(Lys)_n$ under non-ψ conditions. Therefore, methylation of histones may serve to alter the structure of chromatin. At low ionic strength, $[Lys(Me_3)]_n$ and $(Lys)_n$ alter the viscosity of DNA to the same extent between r values of 0.0 and 1.0. In contrast to $(Lys)_n$–DNA, at high ionic strengths, $[Lys(Me_3)]_n$–DNA does not show ψ-type CD spectra. $(Lys)_n$ forms ψ^- structures with $(dA\text{-}dT)_n$ and $(dG\text{-}dC)_n$. $[Lys(Me_3)]_n$ forms ψ^- structures with $(dA\text{-}dT)_n$. Between 0.05 and 0.3 M NaCl, $[Lys(Me_3)]_n$ forms ψ^+ structures with $(dG\text{-}dC)_n$, while between 0.35 and 0.45 M NaCl, it forms a ψ^- structure with $(dG\text{-}dC)_n$. Neither $(Lys)_n$ nor $[Lys(Me_3)]_n$ forms ψ structures with $(dA)_n\cdot(dT)_n$ or $(dG)_n\cdot(dC)_n$. These results, in conjunction with the work of others on reconstitution of nucleosome-like particles from synthetic polynucleotides, suggest that the ability of DNA and histones to form nucleosomes is related to the formation of ψ structures. $(Lys)_n$ binds preferentially to $(dA)_n\cdot(dT)_n$ over $(dA\text{-}dT)_n$. $[Lys(Me_3)]_n$ binds to $(dA)_n\cdot(dT)_n$ and $(dA\text{-}dT)_n$ with equal affinity.

Methylated lysine residues are found in a variety of proteins, including histones H3 and H4 [see Paik & Kim (1980) for a review]. Interesting speculations about the functional effects of methylation of histones have been published (Brandt et al., 1975; Delange & Smith, 1975; Dixon et al., 1975; Paik & Kim, 1972). These speculations were a priori in nature since nothing was known about the effects of methylation on protein conformation or on protein–nucleic acid interactions. Therefore, we are studying the properties of methylated poly(L-lysine), $(Lys)_n$,[1] beginning with the limiting model poly(N^{ϵ},N^{ϵ},N^{ϵ}-trimethyl-L-lysine), $[Lys(Me_3)]_n$ (Granados & Bello, 1979, 1980). We have shown that $[Lys(Me_3)]_n$ stabilizes DNA against thermal denaturation more than $(Lys)_n$ does but is dissociated from nucleic acids at half the salt concentration that $(Lys)_n$ requires (Granados & Bello, 1980). The latter finding suggests that methylation of lysine weakens the electrostatic component of histone–DNA interactions.

Circular dichroism has been used to detect ψ structures in complexes of DNA with histones and polypeptides, in chromatin, and in nucleosomes, for DNA in organic solvents or salt solutions, and for DNA–metal complexes [see Cowman & Fasman (1980) and Shin & Eichhorn (1977) and references therein]. ψ spectra can be negative (ψ^-) or positive (ψ^+) in sign and are characterized by large extrema (some with mean nucleotide residue ellipicity as large as about 10^6 deg cm^2 dmol^{-1}) in the region 260–280 nm. The ψ spectra have been recognized as resulting from asymmetric aggregates. Reich et al. (1980) and Pyatigorskaya et al. (1978) have shown that ψ spectra result from differential scattering of right and left circularly polarized light. The scattering is the result of right- or left-handed superhelix formation, which may occur without a change in secondary structure (Reich et al., 1980; Potaman et al., 1981). We now report on ψ structures that are formed in complexes of $(Lys)_n$ and $[Lys(Me_3)]_n$ with nucleic acids, and we discuss their relationship to chromatin structure.

Experimental Procedure

Materials. (Lys·HBr) (50 000 daltons) was purchased from Sigma Chemical Co., and calf thymus DNA and polynucleotides were purchased from P-L Biochemicals. $[Lys(Me_3)]_n$ was prepared as previously described (Granados & Bello, 1979) except that methylation was done on $(Lys)_n$ in 0.02 M sodium borate (pH 9.2) instead of H_2O. The nucleic acids and polypeptides were dissolved in a pH 8.0 buffer containing 2×10^{-3} M sodium phosphate and 5.0×10^{-4} M EDTA or in EDTA only and were diluted with NaCl prior to direct mixing. The absorption and/or CD spectra of the

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[1] Abbreviations used: $(Lys)_n$, poly(L-lysine); $[Lys(Me_3)]_n$, poly(N^{ϵ},N^{ϵ},N^{ϵ}-trimethyl-L-lysine); EDTA, ethylenediaminetetraacetic acid.

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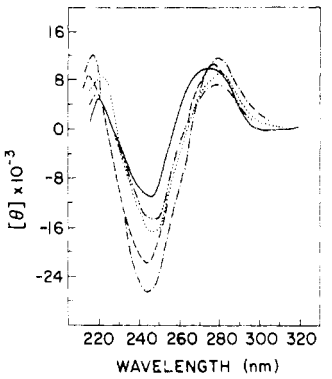

FIGURE 1: CD spectra of $[Lys(Me_3)]_n$–DNA and $(Lys)_n$–DNA in 2.5×10^{-4} M EDTA (pH 8.0). $[Lys(Me_3)]_n$–DNA: $r = 0$ (—), 0.6 (···), 0.8 (---), and 1.0 (–·–·). $(Lys)_n$–DNA: $r = 0.6$ (··–··). r = peptide/nucleotide ratio.

polynucleotides were similar to those in Wells et al. (1970) or references therein, indicating that the polynucleotides were double stranded, except for $(dG)_n$·$(dC)_n$ which was denatured under these conditions. In a buffer containing 0.2 M NaCl, $(dG)_n$·$(dC)_n$ gave a CD spectrum similar to that of Gray & Bollum (1974) for pure double-stranded $(dG)_n$·$(dC)_n$.

Methods. Thermal denaturations were performed with a Cary 219 spectrophotometer equipped for direct recording of absorbance vs. temperature. The temperature of the circulating bath was raised 1 °C/min by using a Neslab EPT-3 temperature programmer. CD measurements were recorded with a Jasco CD-5 instrument, using 1-cm cells. Preparation of complexes and viscosity measurements were done as described earlier (Granados & Bello, 1980).

Results

Interaction with Calf Thymus DNA. Addition of $[Lys(Me_3)]_n$ to a low ionic strength solution of DNA results in a distortion of the CD spectrum of DNA (Figure 1). With an increasing ratio (r) of $Lys(Me_3)$ residues to nucleotide residues (0.0–0.6), the positive extremum near 280 nm decreases and shifts to the red, while the extrema near 220 and 247 nm increase. Similar results were obtained in 0.1 M NaCl (not shown). These results for $[Lys(Me_3)]_n$–DNA are similar to those of Chang et al. (1973) for $(Lys)_n$–DNA. At $r > 0.6$, the positive extremum near 280 nm increases while the negative extremum near 247 nm continues to deepen (Figure 1). In contrast to the results obtained for $[Lys(Me_3)]_n$–DNA at $r > 0.6$, the extremum near 280 nm for $(Lys)_n$–DNA continues to decrease at $r > 0.6$ (Chang et al., 1973). Since the polypeptides alone have CD extrema at and below 220 nm, the effects shown here at 220 nm may arise from the polypeptide and/or the DNA.

The structure of $[Lys(Me_3)]_n$–DNA ($r = 0.5$) as a function of NaCl concentration was studied by CD. Since the $[Lys(Me_3)]_n$–DNA complex is dissociated at about 0.5 M NaCl (Granados & Bello, 1980), this was the highest salt concentration we used. From 0 to 0.5 M salt, only distortions similar to those shown in Figure 1 were observed. This is in contrast to complexes of $(Lys)_n$–DNA which gave ψ spectra at ionic strengths not far from that required for dissociation. We have obtained CD spectra for $(Lys)_n$–DNA complexes somewhat similar to those obtained by Weiskopf & Li (1977) and by Mandel & Fasman (1976) under conditions that produce ψ structures (Figure 2). However, a major difference is that as r increases above 0.25 the positive band near 280 nm increases and shifts to the red, whereas the spectra of Weiskopf & Li (1977) and of Mandel & Fasman (1976) show a decrease in the extremum near 280 nm. The difference may arise from the fact that we use whole complexes which were not centrifuged, while the other authors obtained their data on the complexes present in the supernatant after centrifugation, which may be different in r value and structure.

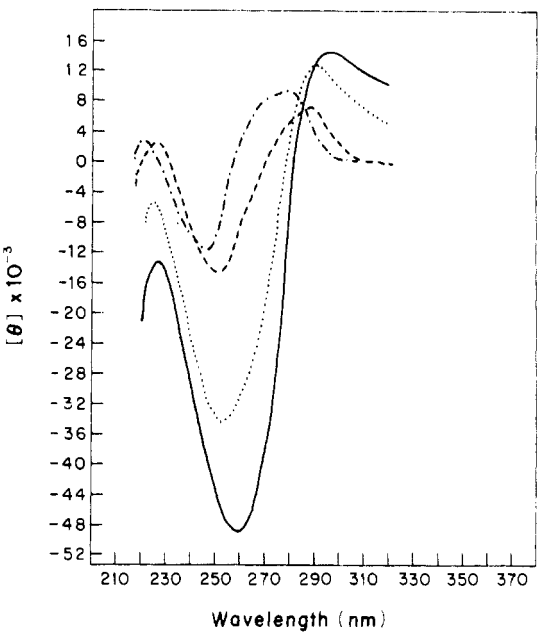

FIGURE 2: CD of $(Lys)_n$–DNA complexes at different r values in 1.0 M NaCl, 1.0×10^{-3} M sodium phosphate (pH 8.0); $r = 0.0$ (–·–), 0.25 (---), 0.50 (···), and 0.75 (—).

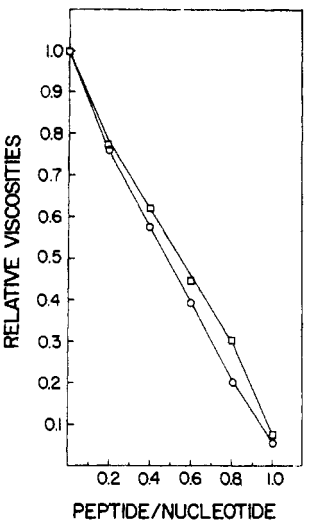

FIGURE 3: Viscosities of $(Lys)_n$–DNA (□) and $[Lys(Me_3)]_n$–DNA (○) relative to unbound DNA as a function of peptide/nucleotide ratio in 2.5×10^{-4} M EDTA (pH 8.0).

Figure 3 shows that the viscosity of DNA is lowered linearly with r for both polypeptides. The results for $(Lys)_n$ are in agreement with those of Zama & Ichimura (1971). The decrease in viscosity of the polypeptide complex with increasing r can be explained by a folding of the complex (Muller & Crothers, 1968).

Interaction with Synthetic Polynucleotides. At 5.0×10^{-6} M nucleotide and $r = 0.75$, neither $(Lys)_n$ nor $[Lys(Me_3)]_n$ distorted the CD spectrum of $(dA)_n$·$(dT)_n$ or of $(dG)_n$·$(dC)_n$ (not shown). [Complexes are formed, however, as shown by fluorescence experiments using dansylated polypeptides (Granados & Bello, 1980).] Under identical conditions, the CD spectra of $(dG\text{-}dC)_n$ and $(dA\text{-}dT)_n$ are markedly altered by $(Lys)_m$ with the formation of ψ^- CD spectra (Figure 4A,B). Figure 4B (inset) also shows that $(Lys)_n$–$(dG\text{-}dC)_n$ begins to

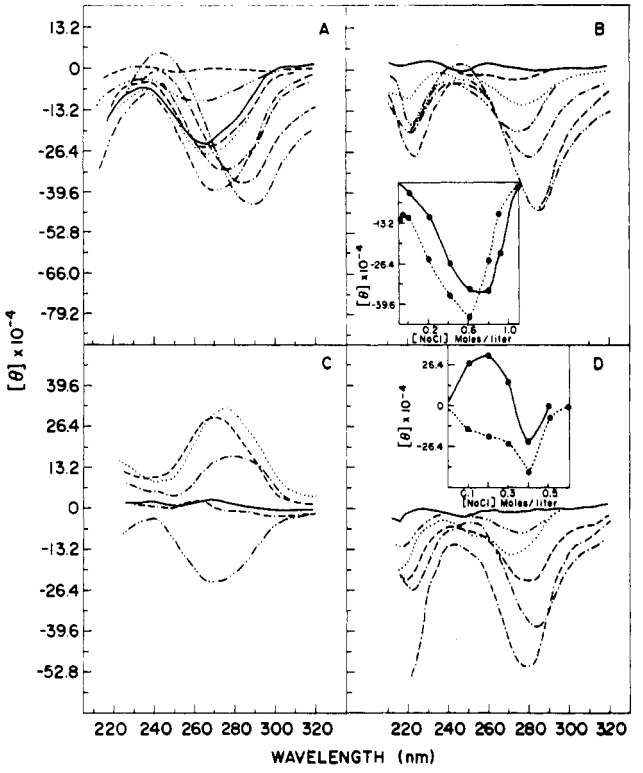

FIGURE 4: CD spectra of polypeptide/polynucleotide complexes at various NaCl concentrations in 1.0×10^{-3} M sodium phosphate and 2.5×10^{-4} M EDTA (pH 7.9). Polynucleotide concentration is 5.0×10^{-6} M and $r = 0.75$. (A) $(Lys)_n$–$(dG\text{-}dC)_n$: NaCl = 0.00 (---), 0.05 (—), 0.10 (···), 0.3 (-·-·), 0.5 (·---), 0.7 (··-··), 0.9 (·· – –), and 1.1 (– – –·) mol/L. (B) $(Lys)_n$–$(dA\text{-}dT)_n$: NaCl = 0.00 and 1.10 (—), 0.10 (---), 0.30 (···), 0.5 (·-·), 0.70 (··-··), 0.90 (—·), and 1.00 (···–) mol/L. (C) $[Lys(Me_3)]_n$–$(dG\text{-}dC)_n$: NaCl = 0.00 (—), 0.10 (---), 0.20 (···), 0.30 (·–), 0.40 (··–), and 0.50 (·– – –·) mol/L. (D) $[Lys(Me_3)]_n$–$(dA\text{-}dT)_n$: NaCl = 0.00 and 0.60 (—), 0.10 (···), 0.20 (---), 0.30 (-·), 0.40 (·-·-·), and 0.50 (··-··) mol/L. (Inset B) $[\theta]_{290}$ (---) and $[\theta]_{265}$ (—) for $(Lys)_n$–$(dG\text{-}dC)_n$ and $(Lys)_n$–$(dA\text{-}dT)_n$, respectively, as a function of NaCl. (Inset D) $[\theta]_{275}$ (—) and $[\theta]_{270}$ (---) for $[Lys(Me_3)]_n$–$(dG\text{-}dC)_n$ and $[Lys(Me_3)]_n$–$(dA\text{-}dT)_n$, respectively, as a function of NaCl.

form ψ^- at lower ionic strength than does $(Lys)_n$–$(dA\text{-}dT)_n$. The return of θ to the low normal value for the polydeoxynucleotides occurs at the salt concentration at which the complexes are dissociated (Granados & Bello, 1980). In sharp contrast to these results, $[Lys(Me_3)]_n$–$(dG\text{-}dC)_n$ gives either a ψ^- or a ψ^+ spectrum, depending on the salt concentration (Figure 4C), ψ^+ from 0.050 to 0.30 M NaCl and ψ^- from 0.35 to 0.45 M NaCl. Similar ψ^+ spectra have been reported for DNA under a variety of conditions [Shin & Eichhorn (1977) and references therein]. To our knowledge, this is the only example of an interconversion between ψ^+ and ψ^- spectra for a DNA–polypeptide complex brought about by a change in ionic strength.

The interactions of $(Lys)_n$ and $[Lys(Me_3)]_n$ with polynucleotides were studied at higher nucleotide concentration ($\sim 5.0 \times 10^{-5}$ M, $r = 0.5$) in the presence of EDTA only or EDTA–0.1 M NaCl. Under both conditions $(Lys)_n$ and $[Lys(Me_3)]_n$ do not distort the CD spectrum of $(dA)_n$·$(dT)_n$. In the presence of 0.1 M NaCl, $(Lys)_n$ distorts the CD spectrum of $(dG)_n$·$(dC)_n$ more than $[Lys(Me_3)]_n$ does (Figure 5A). A somewhat similar distortion for $(dG)_n$·$(dC)_n$ by $(Lys)_n$ was observed by Li et al. (1975). In 0.1 M NaCl, $[Lys(Me_3)]_n$ distorts the spectrum of $(dA\text{-}dT)_n$ more than $(Lys)_n$ does (Figure 5B). In EDTA only, the spectra of $(Lys)_n$–$(dA\text{-}dT)_n$ and $[Lys(Me_3)]_n$–$(dA\text{-}dT)_n$ are similar to that of $(Lys)_n$–$(dA\text{-}dT)_n$ in 0.1 M NaCl (not shown). The results for

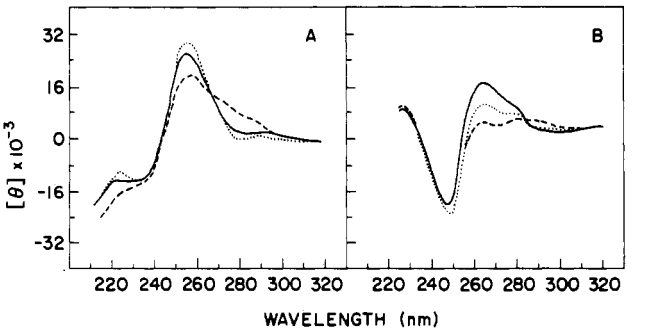

FIGURE 5: CD spectra of polypeptide/polynucleotide complexes in 0.1 M NaCl and 1.0×10^{-3} M sodium phosphate (pH 8.0). Polynucleotide concentration is 5.0×10^{-5} mol/L and $r = 0.5$. (A) $(dG)_n$·$(dC)_n$ (—), $(Lys)_n$–$(dG)_n$·$(dC)_n$ (---), and $[Lys(Me_3)]_n$–$(dG)_n$·$(dC)_n$ (···). (B) $(dA\text{-}dT)_n$ (—), $(Lys)_n$–$(dA\text{-}dT)_n$ (···), and $[Lys(Me_3)]_n$–$(dA\text{-}dT)_n$ (---).

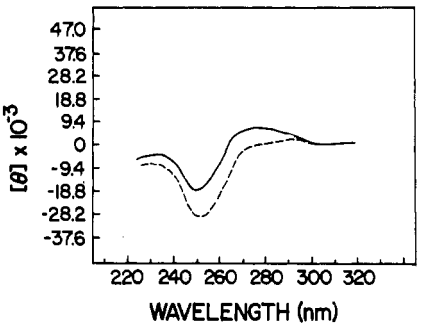

FIGURE 6: CD spectra of polypeptide/polynucleotide complexes in 0.1 M NaCl and 1.0×10^{-3} M sodium phosphate (pH 8.0). Polynucleotide concentration is 3.8×10^{-5} mol/L and $r = 0.5$. $(dG\text{-}dC)_n$ (—), $(Lys)_n$–$(dG\text{-}dC)_n$ (---), and $[Lys(Me_3)]_n$–$(dG\text{-}dC)_n$ (—).

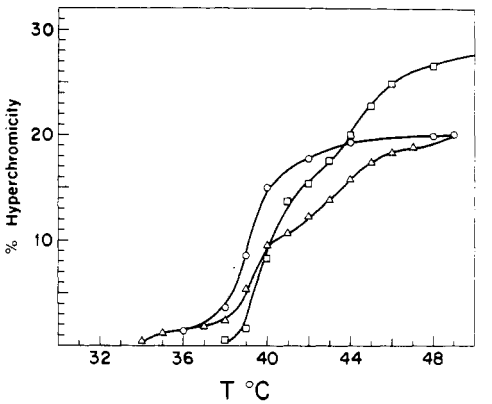

FIGURE 7: Thermal denaturation profiles: $(dA\text{-}dT)_n$ plus $(dA)_n$·$(dT)_n$ (□); $(Lys)_n$ plus $(dA\text{-}dT)_n$ plus $(dA)_n$·$(dT)_n$ (○); $[Lys(Me_3)]_n$ plus $(dA\text{-}dT)_n$ plus $(dA)_n$·$(dT)_n$ (△). Solutions were prepared by gradient dialysis. Hyperchromicity was measured at 260 nm in 0.01 M NaCl and 2.5×10^{-4} M EDTA (pH 7.2). The concentration of each polynucleotide is 5.0×10^{-6} mol/L, and the overall peptide/nucleotide ratio is 0.36. The T_m's shown for the complexes show only the transitions of the unbound polynucleotides.

$(Lys)_n$–$(dA\text{-}dT)_n$ in EDTA only are similar to those of Li et al. (1975). In EDTA, $(Lys)_n$ slightly distorts the spectrum of $(dG\text{-}dC)_n$ while $[Lys(Me_3)]_n$ does not (Figure 6). Thus, under conditions that do not generate ψ spectra, $(Lys)_n$ and $[Lys(Me_3)]_n$ distort the CD spectra of deoxyribopolynucleotides in a somewhat similar manner, although the relative degree of distortion varies with each polynucleotide and salt concentration.

Selectivity Experiments. Leng & Felsenfeld (1966) found that $(Lys)_n$ binds selectively to (A-T)-rich DNA during gradient dialysis. We (Granados & Bello, 1980) obtained a similar result with $[Lys(Me_3)]_n$. However, there has been no

study as to a preference between $(dA)_n \cdot (dT)_n$ and $(dA\text{-}dT)_n$. The results of such an experiment [procedure of Li et al. (1974)] are shown in Figure 7. The thermal denaturation profile for a mixture of $(dA\text{-}dT)_n$ and $(dA)_n \cdot (dT)_n$ shows two transitions, one at 39.8 °C, assigned to $(dA\text{-}dT)_n$, and a second at 44.5 °C, assigned to $(dA)_n \cdot (dT)_n$, corresponding to the T_m's obtained when each is melted separately. The thermal denaturation profile for the $(Lys)_n$–$(dA\text{-}dT)_n$–$(dA)_n \cdot (dT)_n$ mixture is monophasic, with the T_m near 39.4 °C, while that for $[Lys(Me_3)]_n$–$(dA\text{-}dT)_n$–$(dA)_n \cdot (dT)_n$ is biphasic, with the T_m's near 39.5 °C and 43 °C. These results show that $(Lys)_n$ prefers $(dA)_n \cdot (dT)_n$ over $(dA\text{-}dT)_n$, while $[Lys(Me_3)]_n$ shows no preference.

Discussion

Interpretation of CD spectra of DNA has relied to a large extent on the work of Tunis-Schneider & Maestre (1970), who measured the CD spectra of DNA films that were expected to be in the A, B, or C forms (on the basis X-ray fiber studies) and who assigned a spectrum to each form. Chang et al. (1973) suggested that the CD spectra for directly mixed $(Lys)_n$–DNA complexes with $r < 1$ at low ionic strength arise from two classes of base pairs in the complexes, free DNA base pairs which are in the B form and $(Lys)_n$-bound base pairs which are in a conformation that is between the B and C forms. The work of Sprecher et al. (1979) and of Baase & Johnson (1979) indicates very little difference between secondary structures of B and C forms. Chang et al. (1973) pointed out that the spectra of $(Lys)_n$–DNA resemble those of DNA at high NaCl concentrations and that they may be related to dehydration of DNA. The CD changes caused by high salt concentrations have been explained by Hanlon et al. (1975) in terms of the B → C and B → A transitions. They found that C DNA is favored at high ionic strength or low temperature. Mandel & Fasman (1976) suggested that it is inconsistent for the C form to be favored both by high salt and by low temperature since high salt tends to dehydrate DNA while lower temperatures should favor hydration. Chan et al. (1979) suggested that low temperature would increase counterion binding, resulting in more dehydration.

Mandel & Fasman (1976) suggested that the CD changes that occur when $(Lys)_n$ binds DNA do not arise from a B → C transition in DNA but from asymmetric aggregates. Cowman & Fasman (1978, 1980) have suggested that chromatin CD spectra [similar to those observed for $(Lys)_n$–DNA complexes] can be attributed in part to the presence of small amounts of ψ^- structure. This suggestion was based on the observation that difference CD spectra between free DNA and nucleosomal DNA resemble ψ^- spectra. Chang et al. (1973) obtained difference spectra for DNA in high salt vs. low salt and for DNA vs. $(Lys)_n$–DNA, which are similar to those obtained by Cowman & Fasman (1978) for nucleosomes; they attributed the CD changes to dehydration of the DNA. Thus the origin of CD difference spectra obtained at low ionic strengths, which resemble those of ψ^- structures, is still in doubt. The interpretation of CD spectra of DNA has been further complicated by recent work of Zimmerman & Pheiffer (1980) who have suggested that the CD spectral assignment of the C form of DNA by Tunis-Schneider & Maestre (1970) was in error.

From linear infrared dichroism studies, Liquier et al. (1975) concluded that DNA in DNA–$(Lys)_n$ at high humidity is in a form, designated B*, characterized by a change in the orientation of the phosphate group relative to B-form DNA. Prescott et al. (1976) interpreted Raman spectra as showing that the binding of $(Lys)_n$ to DNA at low or high ionic strength does not alter the backbone structure of DNA but alters the interactions between bases, in accord with the finding of Lees & von Hippel (1968) that in DNA–$(Lys)_n$ 25% of the interbase H atoms are very rapidly exchangeable. It appears that the distortion of the DNA CD spectrum induced by the binding of $(Lys)_n$ or $[Lys(Me_3)]_n$ at low ionic strength may be attributed to a number of factors.

Since Sponar & Fric (1972) showed that the magnitude of the ψ^- spectrum given by histone H1–DNA complexes depends on DNA molecular weight, perhaps a difference in polynucleotide molecular weight may be responsible for the formation of ψ structure with $(dG\text{-}dC)_n$ and $(dA\text{-}dT)_n$ but not with $(dG)_n \cdot (dC)_n$ and $(dA)_n \cdot (dT)_n$. Since the sedimentation coefficients (as provided by the vendors) for $(dA)_n \cdot (dT)_n$ and $(dA\text{-}dT)_n$ are 7.2 and 7.9 and for $(dG)_n \cdot (dC)_n$ and $(dG\text{-}dC)_n$ are 11.5 and 9.0, a molecular weight effect seems unlikely. That $(dA)_n \cdot (dT)_n$ forms triple strands at high ionic strength (Arnott & Selsing, 1974) and that $(dG)_n \cdot (dC)_n$ can form quartets of $(dG)_n$ may be related to the fact that these polynucleotides do not form ψ structures under the conditions studied.

Using natural DNA's, Cheng & Mohr (1975) found that the higher the G + C content of the DNA, the larger the magnitude of the ψ^- spectrum induced by salt and poly(ethylene oxide). Sponar & Fric (1972) made a similar finding for the ψ spectra induced by histone H1 in 0.15 M NaCl. Our results for $(Lys)_n$–$(dG\text{-}dC)_n$ and $(Lys)_n$–$(dA\text{-}dT)_n$ at 0.10 M NaCl are compatible with the above since at this ionic strength $(Lys)_n$–$(dG\text{-}dC)_n$ forms ψ^- structures while $(Lys)_n$–$(dA\text{-}dT)_n$ does not. However, at higher ionic strength, both complexes give ψ spectra of comparable magnitude.

The ψ^+ and ψ^- spectra observed for $(dG\text{-}dC)_n$ with $[Lys(Me_3)]_n$ and $(Lys)_n$, respectively, raise questions about differences in secondary and higher order structures. For ψ^-, the B form [Cowman & Fasman (1978) and references therein] and a form intermediate between B and C (Weiskopf & Li, 1977) have been proposed. For DNA in ψ^+ structures A form (Shin & Eichhorn, 1977) and B form (Ong et al., 1976) have been proposed. Although it has been demonstrated that ψ^+ and ψ^- spectra can be generated from right and left twists of supermolecular structures without a change in the secondary structure of the DNA, we may consider the possibility that a change from right-handed (B, C, or A form) to left-handed DNA (Z form) might cause a reversal of twist and a concomitant reversal of the sign of the ψ spectrum. A solution of $(dG\text{-}dC)_n$ at high NaCl concentration undergoes a transition to give a CD spectrum of opposite sign to that at low salt (Pohl & Jovin, 1972). Wang et al. (1979) suggested that the high salt form may be left handed (Z form) and that Z form may be induced in DNA by protein binding. Also, $(dG\text{-}dC)_3$ and $(dG\text{-}dC)_n$ form left-hand helices, as shown by X-ray diffraction (Wang et al., 1979; Arnott et al., 1980). For the ψ^+ and ψ^- spectra of $(dG\text{-}dC)_n$–$[Lys(Me_3)]_n$, there are four possibilities for DNA handedness: right, right; left, left; right, left; left, right.

Although $(Lys)_n$ gives ψ^- spectra with $(dG\text{-}dC)_n$ and $(dA\text{-}dT)_n$ at ~0.1 M NaCl, with calf thymus DNA the ψ^- spectrum appears at ~0.8–0.9 M NaCl. The latter may be the result of mixed sequences in the natural DNA since $(dG)_n \cdot (dC)_n$ and $(dA)_n \cdot (dT)_n$ do not give ψ spectra. The absence of ψ spectra for $[Lys(Me_3)]_n$ with calf thymus DNA likely is the result of dissociation above 0.5 M NaCl.

Selectivity. Woo et al. (1979) speculated that the ammonium group of lysine might hydrogen bond to three sites of a pair of consecutive nucleoside residues (O-1 of deoxyribose

and one site on each base). They noted that with a G-C or C-G sequence such interactions would produce an unfavorable contact between the ammonium group and the N-2 amino group of guanine. No bad contacts were observed with A-T or T-A models. These results, they suggested, may explain the preference of $(Lys)_n$ for (A-T)-rich DNA. Since $[Lys(Me_3)]_n$ binds preferentially to (A-T)-rich DNA (Granados & Bello, 1980) and since the $-NMe_3$ group cannot hydrogen bond, the explanation of Woo et al. (1979) appears to be unlikely.

Relationship to Chromatin. Cowman & Fasman (1978) proposed that adjacent turns of DNA wound around the histone core contribute ψ^- components to the CD spectrum. Rhodes (1979) showed that the octamer of core histones gives nucleosome-like particles with $(dG\text{-}dC)_n$ and $(dA\text{-}dT)_n$ but not with $(dA)_n\cdot(dT)_n$ and $(dG)_n\cdot(dC)_n$. Our finding that $(Lys)_n$ forms ψ structures with $(dA\text{-}dT)_n$ and $(dG\text{-}dC)_n$ but not with $(dA)_n\cdot(dT)_n$ or $(dG)_n\cdot(dC)_n$ suggests that the ability to form nucleosomes is connected with the ability to form ψ structures. Since $[Lys(Me_3)]_n$–$(dG\text{-}dC)_n$ complexes show ψ^+ spectra at physiological ionic strength, lysine methylation of H3 and H4 may serve to regulate the formation of ψ^- structure. The observation that $[Lys(Me_3)]_n$ alters the conformation of some polynucleotides differently from $(Lys)_n$ under conditions where ψ-type structures are not produced (Figures 5 and 6) also points to possible roles for methylated lysines in producing subtle structural differences.

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