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Chromatin Models. Thermal Denaturation Studies of $(\mathrm{Lys}^x,\mathrm{Leu}^y)_n$- and $(\mathrm{Lys})_n(\mathrm{Leu})_m$-DNA Complexes[†]

Edith C. Ong[‡] and Gerald D. Fasman*

ABSTRACT: The structural transitions of $(\mathrm{Lys}^x,\mathrm{Leu}^y)_n$-DNA and $(\mathrm{Lys}^x)_n(\mathrm{Leu}^y)_m$-DNA complexes have been studied by thermal denaturation utilizing simultaneous absorption and circular dichroism (CD) measurements [R. Mandel and G. D. Fasman (1974), *Biochem. Biophys. Res. Commun. 59,* 672]. These complexes are used as models for nucleohistones. At amino acid/nucleotide ratios $r < 1$, the copolymers bind to DNA in a ratio of one amino acid residue per nucleotide, and such binding stabilizes the DNA double helix against thermal denaturation relative to the unbound regions. The leucine residues in the copolymers stabilize the bound portion of the complex against thermal denaturation but to a lesser degree than does poly(L-lysine). This study confirms the hypothesis that absorption melting profiles reflect only the change in secondary structure (helix-coil transition) of DNA. It was found that, in the absence of a higher ordered structure (condensed), the CD melting profile also reflects this same conformational transition, and the melting temperatures, T_m, in CD are equal to those in absorption. However, when a higher ordered structure (tertiary) exists in the complex, then the CD melting profile will be dominated by the structural transitions related to the melting of the higher ordered asymmetric structure in the condensed state, followed by the melting of the secondary structure. Under such circumstances, the T_m obtained from absorption may be slightly different from that of the CD, since only the secondary structural changes are being reflected in absorption. The relevance of these studies to the structure of chromatin is discussed.

The helix-coil transition of the DNA double helix can be induced by acid, base, or temperature (Bloomfield et al., 1974). Thermal denaturation has been used to study the stability of the DNA helix, and the forces which govern this stability. As such it has been used to determine the base composition of the DNA helix (Marmur and Doty, 1962), the conformational properties, and other thermodynamic parameters (Lazurkin et al., 1970).

In an effort to understand the structure of chromatin, thermal denaturation has been widely used to investigate the structure of nucleohistones (Henson and Walker, 1970; Subirana, 1973; Li, 1973; Li et al., 1973; Chang and Li, 1974; Wilhelm et al., 1974a,b; Bram et al., 1974; Adler et al., 1975). Complexes of DNA with synthetic polypeptides, which are assumed to be models for nucleohistones, have been similarly studied (Olins et al., 1967; Privat et al., 1972; Mandel and Fasman, 1974; Li et al., 1974a,b; Santella and Li, 1974; Pinkston and Li, 1974; Sponar et al., 1974, R. Mandel and G. D. Fasman, to be published). The relative strength of binding of proteins or polypeptides to DNA, the nature and extent of such binding, and the effect of such binding on the stability of the DNA double helix can be obtained in this manner.

The thermal denaturation technique with simultaneous absorption and circular dichroism (CD) measurements developed by Mandel and Fasman (1974) is utilized herein to investigate the binding properties of block and random copolymers with different lysine and leucine compositions to DNA, and to study their effect on the structure and the stability of the DNA double helix.

Materials and Methods

Sample Preparation. Calf thymus DNA was prepared according to the procedure of Adler et al. (1971). The various block and random copolymers with different lysine and leucine compositions were previously reported (Snell and Fasman, 1972; Ong et al., 1976). The complexes of DNA with the polypeptides used were made up according to the procedures given in the preceding paper (Ong et al., 1976). The annealed complexes, at the end of a linear gradient dialysis from 1.5 M NaCl × 10^{-3} M EDTA at pH 7.0 ± 0.1 to 0.15 M NaCl × 10^{-3} M EDTA (pH 7.0 ± 0.1), are then exponentially dialyzed twice against 2 l. of 2.5 × 10^{-4} M EDTA solution (pH 7.0 ± 0.1) over a period of 7–8 h. The dialyses of the complexes were performed at 4°. Concentrations of DNA, after dialysis, were determined by perchlorate digestion (Adler et al., 1971).

[†] This is publication No. 1047 from the Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154. *Received May 27, 1975.* This research was supported in part by grants from the U.S. Public Health Service (GM 17533), National Science Foundation (GB 29204X), and the American Cancer Society (P-577).

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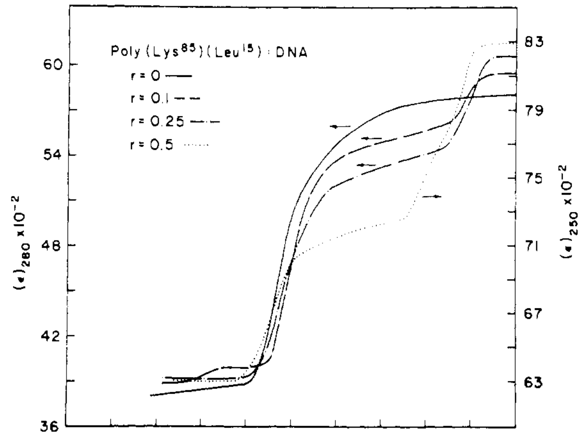

FIGURE 1: Absorption melting curves of DNA (r = 0) and $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$-DNA complexes in 2.5 × 10^{-4} M EDTA (pH 7.0) at different ratios.

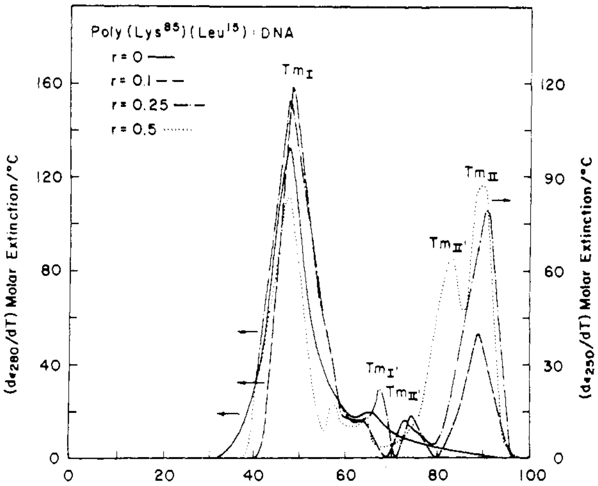

FIGURE 2: Derivative absorption melting profiles of DNA (r = 0) and $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$-DNA complexes in 2.5 × 10^{-4} M EDTA (pH 7.0) at different ratios.

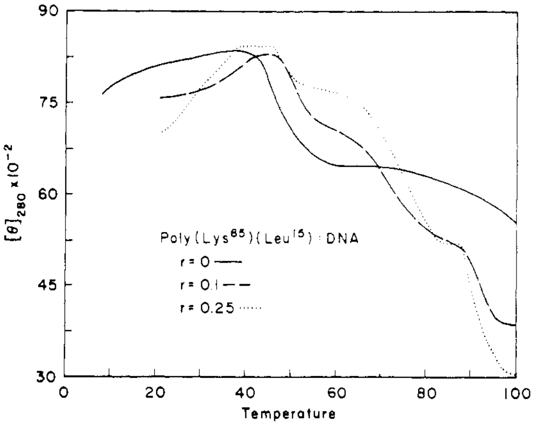

FIGURE 3: Circular dichroism melting curves of DNA (r = 0) and $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$-DNA complexes in 2.5 × 10^{-4} M EDTA (pH 7.0) at different ratios.

Optical Methods. The experimental arrangement for the thermal denaturation studies, using simultaneous absorption and circular dichroism measurements, was that previously described by Mandel and Fasman (1974). The optical density of the samples at the initial temperature was determined on a Cary 14 spectrophotometer at the desired wavelength, and a calibration curve of the optical density vs. CD dynode voltage has been previously obtained. The samples were heated at a rate of 0.3–0.4°/min, and the absorbance, ellipticity, and temperature are simultaneously recorded at the desired wavelength. The data are subsequently processed on a PDP-10 computer with a Calcomp plotter, and both the absorption and circular dichroism melting curves and their respective derivative curves are plotted as described in Mandel and Fasman (1974).

Results

Complexes of DNA with $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$.[1] The absorption and circular dichroism melting profiles and their corresponding derivative melting curves for the complexes of DNA with the block $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$ copolymer at different ratios are given in Figures 1–6. For the purpose of comparison, the absorption melting profiles for DNA alone (r = 0) and for the complexes at different ratios are seen in Figure 1. The absorption spectra of the complexes at 280 nm for r = 0.1 and 0.25, and at 250 nm for r = 0.5 are corrected for scattering effects by normalizing to the DNA value (r = 0) at the initial temperature. The derivatives of the absorption melting curves are given in Figure 2 for these same complexes and more clearly depict the melting temperature for the bimodal transition of the DNA complexes. Two major melting bands ($T_{\mathrm{mI}} + T_{\mathrm{mII}}$) with one or two intermediate minor bands (T_{mI}', + T_{mII}') are observed for the complexes. T_{mI}, the temperature of the first melting band for the complexes at different ratios, is equal to that of the melting temperature (T_{mI}) for DNA alone (r = 0), where T_{mI} = 47 ± 1°. This band corresponds to the melting of the free DNA in regions of DNA unbound by the copolymer. A second major melting band, with T_{mII} = 90 ± 1°, was found for the complexes, and is absent for the melting of DNA alone (r = 0). This band would therefore correspond to the melting of DNA regions protected or bound by the copolymers.

A minor melting band (T_{mI}') at ~65–67° was observed for the complexes and for DNA alone, and may correspond to the melting of the GC-rich region of the free DNA (Felsenfeld and Sandeen, 1962). Another minor melting band, with T_{mII}' at ~73, 75, and 83° was found for the complexes at r = 0.1, 0.25, and 0.5, respectively, and was absent for the melting of DNA alone (r = 0). This band may reflect the melting of DNA regions covered by the leucine sequence, which may have a different binding site from the lysine-bound regions.

The fraction of free (A_{f}) and bound (A_{b}) DNA can be estimated from the integrated area under the bimodal melting bands (Figure 2), where $A_{\mathrm{f}} + A_{\mathrm{b}} = 1$. If the areas of the intermediate melting bands T_{mI}' and T_{mII}' are included in the integrated area for the fractions of free (A_{f}) and bound (A_{b}) DNA, respectively, then A_{b} is found to be in very good agreement with the input ratio for r_{b} = (Lys + Leu)/PO_4 (see Table I). This indicates that the leucine residues are also involved in stabilizing the DNA helix against thermal denaturation relative to free DNA.

The width of the melting band (ΔT), which is taken at the band half-maximum, reflects the sharpness of the tran-

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Abbreviations used are: PEO, polyethylene oxide. All amino acids are L. Histone nomenclature from Bradbury (1975): H1 (F1, Ia); H2A (F2a2, IIb1); H2B (F2b, IIb2); H3 (F3, III); H4 (F2a1, IV); H5 (F2C, 5 or V).

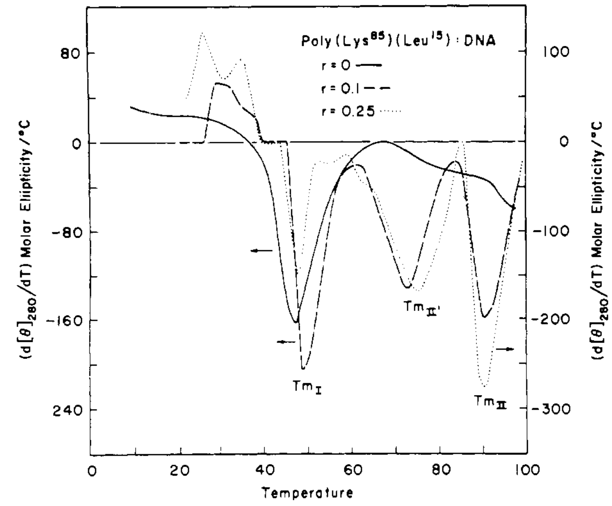

FIGURE 4: Derivative circular dichroism melting profiles of DNA ($r = 0$) and $(Lys^{85})_n(Leu^{15})_m$-DNA complexes in 2.5×10^{-4} M EDTA (pH 7.0) at different ratios.

sition and can be related to the heterogeneity of the DNA base sequence involved in the thermal transition in question (Bloomfield et al., 1974). It was found that the width of the first melting band (ΔT_I) for the complexes at different r values (Figure 2) is approximately equal to the width of the DNA ($r = 0$) melting band, where $\Delta T_{I(r \neq 0)} = \Delta T_{I(r=0)} = 10 \pm 1°$. This indicates that the base sequence of the unbound DNA in the complex is of the same heterogeneity as DNA alone ($r = 0$), which implies that a preferential binding of the copolymer, $(Lys^{85})_n(Leu^{15})_m$, to the A-T rich regions of DNA is not observed for the ratios investigated. The fact that T_{mI} of the complex at different r values is equal to the melting temperature of DNA alone ($r = 0$) also agrees with the above argument that no preferential binding is observed for these ratios.

The results of Pinkston and Li (1974) on the thermal denaturation of $(Lys^{40},Ala^{60})_n$-DNA complexes showed that T_{mI} of the complex at $r < 0.5$ Lys/DNA is equal to that of DNA ($r = 0$), but is considerably increased at higher r. Santella and Li (1974) have found an increase in T_{mI} relative to that of DNA ($r = 0$) for $(Lys^{50},Tyr^{50})_n$-DNA complexes even at $r < 0.2$ Lys/DNA. Li et al. (1974a,b) observed a slight increase in T_{mI} with increasing r for poly(L-lysine)-DNA complexes, and showed that $(Lys)_n$ binds preferentially to A-T rich DNA, which agrees with the titration experiments of Leng and Felsenfeld (1966).

The circular dichroism spectra of the $(Lys^{85})_n(Leu^{15})_m$-DNA complex, at $r = 0.1$ and 0.25, are very similar to the spectra of B-form DNA in solution (Ong et al., 1976). The circular dichroism melting profiles of these complexes and their respective derivative melting curves are shown in Figures 3 and 4, together with the melting curves for DNA alone ($r = 0$) at 280 nm. The "premelting" transition, which is characterized by a broad transition curve with slowly increasing ellipticity values at 280 nm prior to the helix-coil transition of DNA, is more pronounced for the complexes than for DNA alone (Figure 3). Such a premelting transition of DNA has been the subject of several investigations (see discussion below) and has also been observed by Mandel and Fasman (1974) in their studies of $(Lys^{84.5},Val^{15.5})_n$-DNA complexes. The interpretations of the premelting transition will be considered later in the discussion.

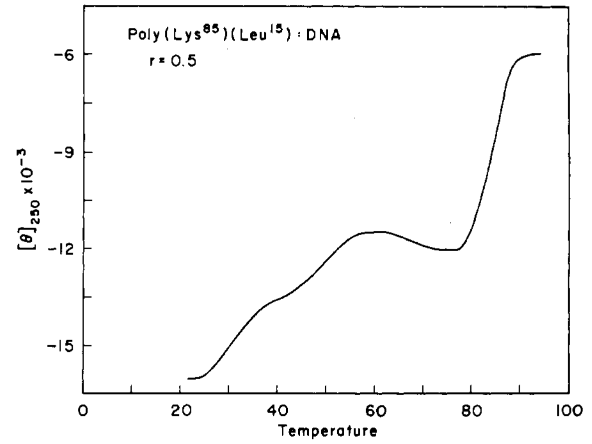

FIGURE 5: Circular dichroism melting curve of $(Lys^{85})_n(Leu^{15})_m$-DNA complex in 2.5×10^{-4} M EDTA at $r = 0.5$.

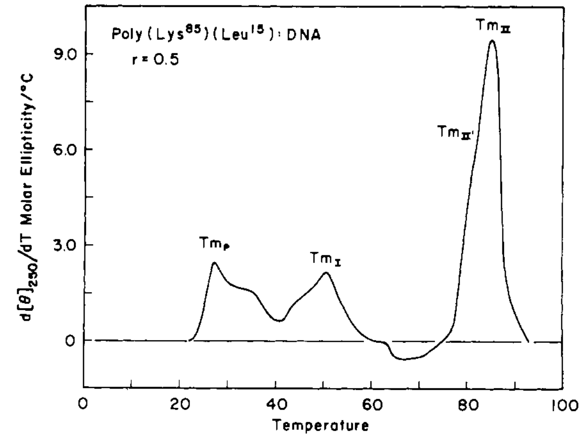

FIGURE 6: Derivative circular dichroism melting profile of $(Lys^{85})_n(Leu^{15})_m$-DNA complex in 2.5×10^{-4} M EDTA at $r = 0.5$.

Examination of the CD melting profiles (Figures 3 and 4) above 40° clearly reveals three melting transitions for the complex at $r = 0.1$ and 0.25, with $T_{mI} = 48 \pm 1°$, $T_{mII}' = 74 \pm 1°$, and $T_{mII} \simeq 91 \pm 1°$, and only a single major melting transition with $T_{mI} = 47 \pm 1°$ for DNA alone ($r = 0$). The T_m's obtained from the CD melting profiles are in very good agreement with the corresponding T_m's obtained from the absorption melting profiles (Table I), and the presence of T_{mII}' is more clearly revealed in the CD profiles.

The CD of the $(Lys^{85})_n(Leu^{15})_m$-DNA complex at $r = 0.5$, in 2.5×10^{-4} M EDTA (pH 7.0 ± 0.1), is characterized by a small positive band at ~285 nm ($[\theta]_{285} \simeq 3500$ deg cm²/dmol of nucleotide) and a large negative band at ≃250 nm with $[\theta]_{250} \simeq 16000$ deg cm²/dmol of nucleotide. It is believed that such spectra reflect a structure of DNA in the condensed state similar to that of the Ψ structure (Jordan et al., 1972; Ong et al., 1976). The CD melting profile of such a complex, monitored at 250 nm, and its corresponding derivative melting curve are shown in Figures 5 and 6. Here, a large "premelt", depicted by an initial decrease in magnitude at 250 nm with increasing temperature, in the beginning of the melting transition, was observed. The slope and magnitude of this premelting transition are approximately equal to that of the first melt, which has a $T_{mI} \simeq 50 \pm 1°$, slightly higher than its corresponding absorption melt. Immediately following the first melt, and

Table I: Thermal Denaturation Parameters for DNA–Polypeptide Complexes.[a]

		°C			°C		°C					
Copolymer	r	T_{mI}(Abs)	T_{mI}(CD)	ΔT_{I}[f](Abs)	T_{mII}'(Abs)	T_{mII}'(CD)	T_{mII}(Abs)	T_{mII}(CD)	ΔT_{II}(Abs)	r_b[e]	A_f[g]	A_b[h]
$(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$	0.1	47 ± 1	48 ± 1	10 ± 1	~73	74 ± 1	90 ± 1	91 ± 1	7 ± 0.5	0.12	0.83	0.17
	0.25	47 ± 1	48 ± 1	11 ± 1	~75	74 ± 1	90 ± 1	91 ± 1	7 ± 0.5	0.29_4	0.70	0.30
	0.5	47 ± 1	50 ± 1	9 ± 1	~83	~81	90 ± 1	85 ± 1	7 ± 0.5	0.59	0.46	0.54
$(\mathrm{Lys}^{77})_n(\mathrm{Leu}^{23})_m$	0.5	46 ± 1	46 ± 1	10 ± 1	~65, 71 ± 2	65 ± 2	81 ± 2	82 ± 2	7.5 ± 0.5	0.65	0.56	0.44
$(\mathrm{Lys}^{84}, \mathrm{Leu}^{16})_n$	0.5	44 ± 2	49 ± 1	10 ± 1			87 ± 1	84 ± 1	9 ± 1	0.59	0.43	0.57
$(\mathrm{Lys}^{68}, \mathrm{Leu}^{32})_n$	0.1	49 ± 1		9 ± 1			85 ± 1	85 ± 1	6 ± 0.5	0.15	0.88	0.12
	0.25	49 ± 1		10 ± 1			85 ± 1	83 ± 1	7.5 ± 0.5	0.37	0.61	0.39
$(\mathrm{Lys})_n$[b]	0.3	48 ± 1	48 ± 1		96.3	95.0		100				
	0.6	51 ± 1			97.2	94.5		100				
$(\mathrm{Lys}^{40}, \mathrm{Ala}^{60})_n$[c]	0.24	48					93			0.6		
	0.48	48					93			1.2		
	0.72	53					93			1.8		
	0.12	53					93			2.8		
$(\mathrm{Lys}^{50}, \mathrm{Tyr}^{50})_n$[d]	0	61								0	1.00	
	0.18	67					90			0.36	0.9	0.1
	0.36	68					89			0.72	0.66	0.28
	0.54	72					90			1.08	0.46	0.47
	0.72	75					90			1.44	0.22	0.49
DNA	0	47 ± 1		10 ± 1								

[a] 2.5×10^{-4} *M* EDTA (pH 7.0). [b] R. Mandel and G. D. Fasman, to be published. [c] Direct mix complex in 2.5×10^{-4} M EDTA (pH 8) (Pinkston and Li, 1974). [d] Direct mix complex in 10^{-3} M Tris (pH 6.8) (Santella and Li, 1974). [e] r_b = amino acids (moles)/DNA, i.e., Lys + Leu, Lys + Ala, Lys + Tyr. [f] ΔT_1 (Abs) = width of the derivative absorption curve taken at half-maximum of the first melting band. [g] A_f = the ratio of the integrated area under the derivative of the first melting band to the total area of the bimodal melting bands. [h] A_b = the ratio of the integrated area under the derivative of the second melting band to the total area of the bimodal melting bands.

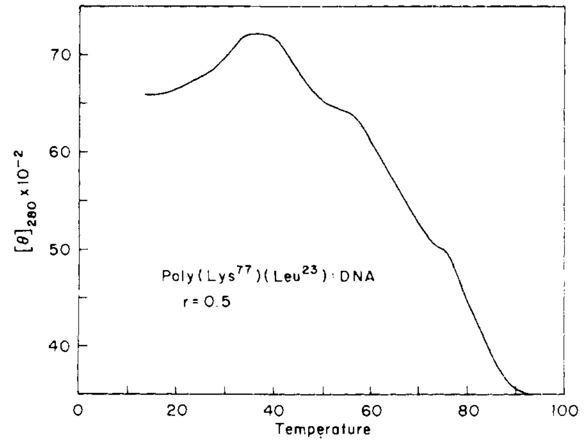

FIGURE 7: Circular dichroism melting curve of $(\mathrm{Lys}^{77})_n(\mathrm{Leu}^{23})_m$–DNA complex in 2.5×10^{-4} M EDTA at $r = 0.5$.

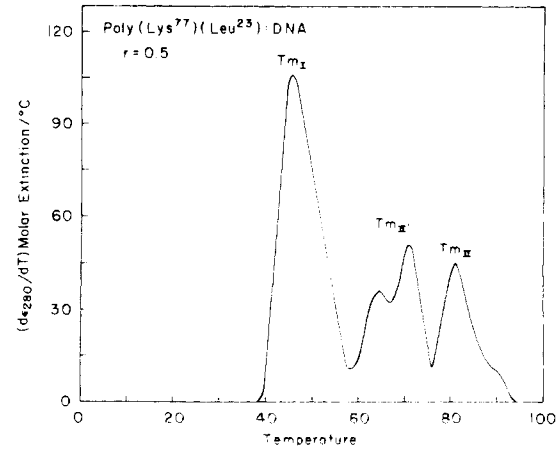

FIGURE 8: Derivative absorption melting profile of $(\mathrm{Lys}^{77})_n(\mathrm{Leu}^{23})_m$–DNA complex in 2.5×10^{-4} M EDTA at $r = 0.5$.

prior to the second melt, there is a very small increase in magnitude at 250 nm with increasing temperature. However, the second melt, which corresponds to the melting of the bound segments of DNA, has a $T_{\mathrm{mII}} \simeq 85 \pm 1°$, considerably lower than its corresponding absorption melt (see Table I), and the slight shoulder at ~81° may correspond to the T_{mII}' in absorption.

Complexes of DNA with $(Lys^{77})_n(Leu^{23})_m$. The CD spectra for the complex of DNA with block polypeptide, $(\mathrm{Lys}^{77})_n(\mathrm{Leu}^{23})_m$, at $r = 0.5$ in 2.5×10^{-4} M EDTA buffer (pH 7.0 ± 0.1) are characterized by a conservative spectrum with $[\theta]_{280} \simeq 6800 \pm 300$ and $[\theta]_{245} \simeq -7000 \pm 300$ (Ong et al., 1976). The CD melting profile at 280 nm and the derivative of the absorption melting profile are shown in Figures 7 and 8. The CD melting profile (Figure 7) is similar to that obtained for $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$–DNA complexes at $r = 0.1$ and 0.25, but with $T_{\mathrm{mI}} \simeq 46 \pm 1°$, $T_{\mathrm{mII}}' \simeq 65 \pm 2°$, and $T_{\mathrm{mII}} \simeq 82 \pm 2°$. The absorption melting profile gives three distinct transitions with $T_{\mathrm{mI}} \simeq 46 \pm 1°$, $T_{\mathrm{mII}}' \sim 68°$, and $T_{\mathrm{mII}} \simeq 81 \pm 2°$ (not shown). However, the absorption derivative curve (Figure 8) shows a splitting of T_{mII}' into a shoulder at 65 ± 2° and a peak at 81 ± 2° Here again, the T_{m}'s in absorption and CD profiles are in good agreement with each other. The fraction of the bound DNA calculated from the integrated area under T_{mII}' and T_{mII} of the derivative absorption curve appears to be lower than the input ratio of r_b = (Lys + Leu)/$\mathrm{PO_4}^{3-}$ (see Table I). This may be due to the presence of a very long sequence of leucine residues (hydrophobic region) at one end of the copolymer which is capable of self-association at high salt and prevents extensive interaction with DNA.

Complexes of DNA with $(Lys^{84}, Leu^{16})_n$. The complex of DNA with the random copolymer, $(\mathrm{Lys}^{84}, \mathrm{Leu}^{16})_n$, in 2.5×10^{-4} M EDTA (pH 7.0 ± 0.1) at $r = 0.5$, gives a nonconservative circular dichroism spectrum similar in shape to the Ψ spectra (Jordan et al., 1972) with a minimum ellipticity at 270 nm, $[\theta]_{270} = -19\,500 \pm 800$ (Ong et al., 1976). The CD melting profile and the derivative of its absorption

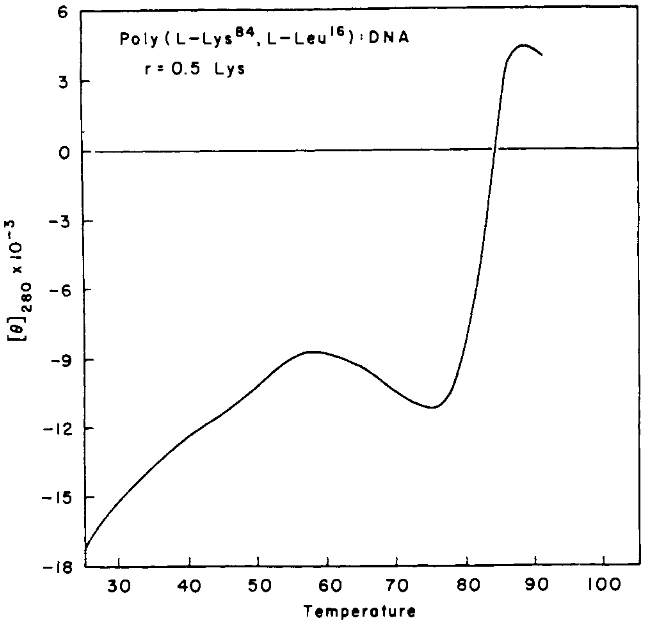

FIGURE 9: Circular dichroism melting curve of $(\text{Lys}^{84},\text{Leu}^{16})_n$–DNA complex in 2.5×10^{-4} M EDTA at $r = 0.5$.

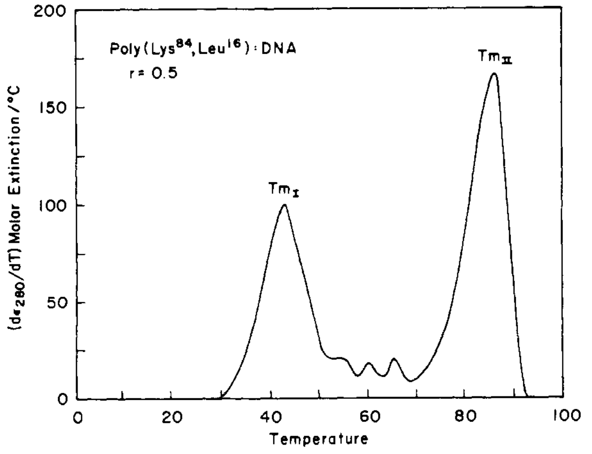

FIGURE 10: Derivative absorption melting profile of $(\text{Lys}^{84},\text{Leu}^{16})_n$–DNA complex in 2.5×10^{-4} M EDTA at $r = 0.5$.

melting profile at 280 nm are seen in Figures 9 and 10. Again there is a large premelt in the CD profile (Figure 9) followed by the first melt with $T_{\text{mI}} = 49 \pm 1°$. However, T_{mI} in absorption equals 44 ± 2° (Figure 10), slightly lower than that of native DNA. T_{mII}, which corresponds to the melting temperature of the bound regions of DNA, is 87 ± 1° in absorption and 84 ± 2° in CD (Table I). The slight discrepancy between the melting temperatures in CD and absorption may be a result of the difference in sensitivity between the two techniques to the different processes involved in thermal denaturation, i.e., unwinding or unfolding of DNA, strand separation, loop formation of the melted regions, and the subsequent loss of structure to the coil state after complete strand separation has taken place.

The fraction of bound DNA calculated from the second peak of the derivative absorption melts (Figure 10) agrees quite well with the input ratio of r_b = (Lys + Leu)/DNA phosphates.

Complexes of DNA with $(\textit{Lys}^{68},\textit{Leu}^{32})_n$. The absorption and CD melting profiles at 280 nm for the complexes of DNA with the random copolymer, $(\text{Lys}^{68},\text{Leu}^{32})_n$, at r = 0.1 and 0.25 are shown in Figures 11–13. The absorption melting profile (Figure 11) reflects two major melting transitions with $T_{\text{mI}} = 50 \pm 1°$ corresponding to the free DNA melt and $T_{\text{mII}} = 85 \pm 1°$ corresponding to the melting of the bound portion of DNA. A minor band with $T_{\text{mI}}' \sim 65°$ is also revealed (Figure 11). The relative area under the derivative curves (Figure 11) gives the fractions of bound and unbound DNA, and corresponds well to the input ratio of copolymer/DNA (see Table I).

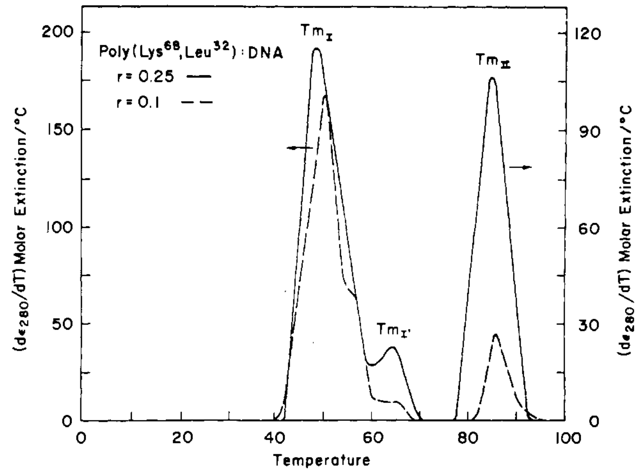

FIGURE 11: Derivative absorption melting profiles of $(\text{Lys}^{68},\text{Leu}^{32})_n$–DNA complexes in 2.5×10^{-4} M EDTA at different ratios.

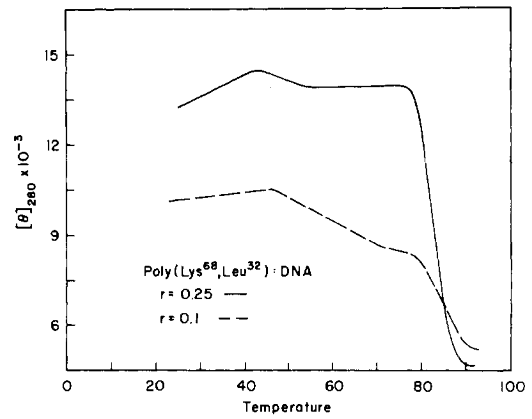

FIGURE 12: Circular dichroism melting curves of $(\text{Lys}^{68},\text{Leu}^{32})_n$–DNA complexes in 2.5×10^{-4} M EDTA at different ratios.

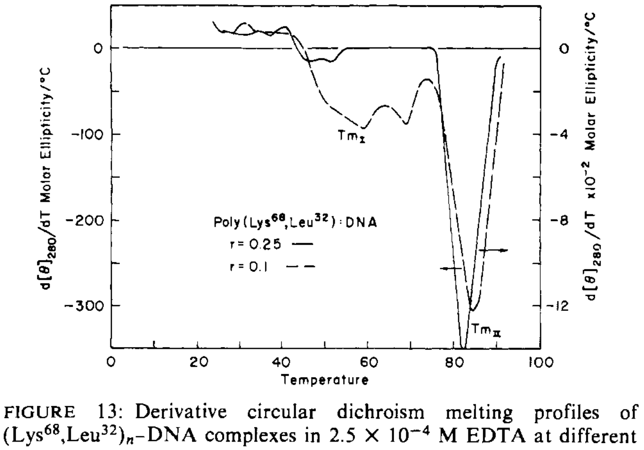

FIGURE 13: Derivative circular dichroism melting profiles of $(\text{Lys}^{68},\text{Leu}^{32})_n$–DNA complexes in 2.5×10^{-4} M EDTA at different ratios.

The CD spectra of these complexes are characterized by a large positive band at 270 nm and a small negative band at ~240 nm and are believed to represent a structure of DNA in the condensed state (Ong et al., 1976). The CD melting profiles, at 280 nm (Figures 12 and 13), show a very slight premelt, followed by a small and broad transition with a very poorly defined T_{mI}, and then a very sharp melting transition with $T_{\mathrm{mII}} = 83 \pm 2°$, which corresponds to the collapse of the DNA which binds the copolymer.

Discussion

The absorption and circular dichroism melting curves for the complexes of DNA with block, $(\mathrm{Lys}^x)_n(\mathrm{Leu}^y)_m$, and random, $(\mathrm{Lys}^x,\mathrm{Leu}^y)_n$, copolymers of different amino acid compositions and different ratios are summarized in Table I. The melting curves for these complexes are irreversible, and thus may be viewed as representing a "frozen" version of the equilibrium prevailing during the annealing process. Thus it is presumed that the polypeptide ligands cannot move from one location to another during the melting process, and/or that the binding of the ligands is cooperative, thus leaving some "naked" DNA regions.[2]

It can be seen that the fractions of free and bound DNA, calculated from the respective absorption melting curves for each complex (Li and Bonner, 1971), agree quite well with the input ratio if one takes into consideration the leucine as well as the lysine residues per DNA phosphate. (An exception is the complex with block $(\mathrm{Lys}^{77})_n(\mathrm{Leu}^{23})_m$ copolymer which may be considerably aggregated.) This would imply that all the amino acid residues of the synthetic polypeptides, charged and uncharged hydrophobic residues, are bound to the DNA nucleotides on a 1:1 basis, i.e., one amino acid residue per nucleotide. These bound residues stabilize the double helical structure of DNA against thermal denaturation relative to the free or unbound DNA, as depicted by the bimodal melting curves of the complexes. This also agrees with the work of Mandel and Fasman (1974), where it was found that a 1:1 ratio of amino acid residue bound per nucleotide phosphate existed in their thermal denaturation studies of $(\mathrm{Lys}^{84.5},\mathrm{Val}^{15.5})_n$-DNA complexes (at an input ratio of $r = 0.25$ Lys/PO$_4$, which corresponds to $r_b = (0.3\ \mathrm{Lys} + \mathrm{Val})/\mathrm{PO_4}$). Li (1973) had previously found that a one amino acid residue bound per nucleotide relationship exists in poly(L-lysine)-DNA complexes at $r \leq 1.0$ using the experimental data observed by Olins et al. (1967) and Shih (1969). Li et al. (1974b) observed a biphasic melting for the poly(L-lysine)-DNA complex at an input ratio of 0.6 lysine residue per nucleotide, and found that 57% of the total hyperchromicity occurs at T_{mII}, the lysine bound fraction. This study also demonstrated that a small 13% shoulder existed on the derivative curve, T_{mI}, which probably represented a G + C rich unbound area in the DNA. However, in the thermal denaturation studies of $(\mathrm{Lys}^{50},\mathrm{Tyr}^{50})_n$-DNA complexes, Santella and Li (1974) have shown that there are two amino acid residues bound per nucleotide of DNA, or one lysine and one tyrosine residue per nucleotide. In a recent study, Pinkston and Li (1974) found that there is approximately one lysine residue per nucleotide in the bound region of $(\mathrm{Lys}^{40},\mathrm{Ala}^{60})_n$-DNA complex.

However, these two studies should be viewed with caution as these complexes were made by direct mixing rather than by reconstitution (i.e., gradual lowering of the ionic strength). It is well known that attempts to reassemble chromatin without gradual lowering of the ionic strength have met with gross failure. Reconstitution by gradient dialysis or stepwise lowering of the ionic strength has met with greater success (Bekhor et al., 1969; Gilmore and Paul, 1970; Chae, 1975).

The thermal denaturation results of Table I will be discussed in two parts. (1) *Polypeptide-DNA complexes which give a conservative CD spectrum, similar to that of native DNA in solution.* Such CD spectra are observed with complexes of DNA with the block copolymer $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$ at $r = 0.1$ and 0.25, and with the block copolymer $(\mathrm{Lys}^{77})_n(\mathrm{Leu}^{23})_m$ at $r = 0.5$. (2) *Complexes which give altered CD spectra* relative to native DNA in 2.5×10^{-4} M EDTA solution (Ong et al., 1976). Such spectra are observed with the complexes of DNA with the block copolymer $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$ at $r = 0.5$, with the random copolymer $(\mathrm{Lys}^{84},\mathrm{Leu}^{16})_n$ at $r = 0.5$, and with the random copolymer $(\mathrm{Lys}^{68},\mathrm{Leu}^{32})_n$ at $r = 0.1$ and 0.25. These spectra are believed to result from the condensed state of DNA.

The structure of the condensed state of DNA for the complexes herein has been thoroughly discussed by Ong et al. (1976). It was suggested that the Ψ structure observed for $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$-DNA and $(\mathrm{Lys}^{84},\mathrm{Leu}^{16})_n$-DNA complexes at $r = 0.5$ is similar to that of the structure of DNA in polyethylene oxide and salt solution (Jordan et al., 1972; Cheng and Mohr, 1974). These structures are believed to result from the condensation of DNA into a chain folded, highly ordered asymmetric superstructure similar to that of the cholesteric liquid crystals. It was shown that the A-like spectra observed for $(\mathrm{Lys}^{68},\mathrm{Leu}^{32})_n$-DNA complexes at $r = 0.1$ and 0.25 can also be derived from the same condensation phenomena. The difference between the Ψ-type and the A-like type structures may be due to the difference in the geometry of packing or in the asymmetry of these superstructures.

(*1*) *Polypeptide-DNA Complexes with Conservative CD Spectrum.* It can be seen that the shape of the CD melting curves for $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$-DNA complexes at $r = 0.1$ and 0.25, and the $(\mathrm{Lys}^{77})_n(\mathrm{Leu}^{23})_m$-DNA complexes at $r = 0.5$, monitored at λ 280 nm, are similar (Figures 3 and 7). The CD melting profiles for these complexes show at least three melting transitions plus a premelt. The premelts of the $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$-DNA complexes (Figure 3) are larger than that of pure DNA ($r = 0$) in the order, $r = 0.25 > r = 0.1 > r = 0$. The same ellipticity value is approached at the end of each premelt at 280 nm, before undergoing the first melt. Mandel and Fasman (1974) had also observed a larger premelt for the $(\mathrm{Lys}^{84.5},\mathrm{Val}^{15.5})_n$-DNA complex at $r = 0.25$ than that for DNA ($r = 0$) in their CD melting curve monitored at 280 nm.

There have been several investigations stating that the CD melting transition of nucleoprotein lacks the premelting transition found in DNA alone (Henson and Walker, 1970; Wilhelm et al., 1974a,b; Ramm et al., 1972). However, the transition is restored on removing H-1 (a small premelt, Henson and Walker, 1970), non-histone proteins, or when only the lysine rich histones, H2B and H2A, have been removed (Wilhelm et al., 1974a,b). The premelting transition is also observed when only H1 is present in the nucleoprotein complex (Wilhelm et al., 1974a,b). It was further demonstrated that the histones undergo a cooperative disruption of a large amount of α helix prior to the DNA melt (Wilhelm et al., 1974b) while further changes in histone structure occur after the premelt (Ramm et al., 1972). It was

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[2] We are indebted to a referee for discussing this point.

concluded that the size of the premelt is proportional to the amount of the histones removed. These studies indicated that the repression of the premelt was due to various histone-histone interactions.

The results from a high resolution absorption melting study of histone-DNA complexes were interpreted by Bram et al. (1974) as indicating that the premelting phenomena may be due to the true melting of AT-rich regions in DNA. However, the premelting transition of the DNA complexes herein, observed in the CD spectra at 280 nm, is not observed in absorption studies, and it is assumed that the "premelting" phenomena observed by Bram et al. (1974) pertained to the melting of DNA in the beginning of the T_{mI} band, and is not the same premelting transition discussed here.

The phenomena of premelting in the denaturation of DNA has been observed, since 1963, by various physical-chemical techniques [viscosity change—Tikhonenko et al., 1963; Bartl and Boublick, 1965; Utiyama and Doty, 1971; circular dichroism and optical rotatory dispersion—Brahms and Mommaerts, 1964; Samejima and Yang, 1965; Henson and Walker, 1970; Luck et al., 1970; Ramm et al., 1972; Palacek and Fric, 1972; Studdert et al., 1972; Wilhelm et al., 1974a,b; oscillographic polarography—Palacek, 1966; Palacek and Fric, 1972; raman spectroscopy—Erfurth and Peticolas, 1975]. A similar behavior has been reported in the melting of poly[d(A-T)] by viscosity measurements (Spatz and Baldwin, 1965). Although numerous explanations have been offered, there has been no satisfactory theory to date to adequately explain these observations.

The studies by Gennis and Cantor (1972) on the CD premelt behavior of DNA tend to specifically rule out the possibility that premelt is due to a conformational change of B-form DNA to the A-form. They have also shown that the largest premelt occurs for poly[d(A-T)] while the smallest change occurs for poly[d(G-C)]. Studdert and Davis (1974) calculated the CD of poly[d(A-T)] and found a large decrease in the positive band at 280 nm upon increasing the winding angle (θ) from 36.5 to 38.6°, and only a very slight decrease of the 280-nm band for poly[d(G-C)] upon increasing θ from 36.5 to 40°. Johnson and Tinoco (1969) suggested that the bases of DNA in the premelting stage may be tilted in preparation to disrupt the H-bonds, since their calculation shows that the tilting of DNA bases could result in a large CD change.

A characteristic of the DNA complexes with $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$ at $r = 0.1$ and 0.25, and with $(\mathrm{Lys}^{77})_n(\mathrm{Leu}^{23})_m$ at $r = 0.5$, which give CD spectra similar to that of native DNA, is that their T_{m}'s obtained from absorption are equal to that in CD to within ±1° (see Table I), and that their T_{mI}, which corresponds to the melting of free DNA, is similar to the T_{mI} of DNA alone ($r = 0$). Mandel and Fasman (1974) also reported similar T_{m} values for CD and absorption denaturation in their study of the $(\mathrm{Lys}^{84.5},\mathrm{Val}^{15.5})_n$-DNA complex at $r = 0.25$, where the CD of the complex also resembles that of B-form DNA in solution. The T_{mI} of the complex is also identical with the T_{m} of DNA alone.

An examination of Figures 3 and 4 reveals that the bound fraction of DNA cannot be quantitatively obtained from the area under the derivative CD bands. However, the percent of free DNA in the complex, which is depicted by the percent decrease in the ellipticity value at 280 nm for the T_{mI} band of the complex relative to the T_{mI} band of DNA alone, is in qualitative agreement with the input ratio of the polypeptide in the complex. The inability to quantitatively correlate the ratio of the CD melting profile of the complex into free and bound fractions of DNA indicates that the parameters affecting the CD melting profiles are more complicated than that of the absorption. While hyperchromicity is mainly affected by stacking interactions between the DNA bases, CD changes can be affected by the changes in the relative distance of the bases to the helix axis, the changes in tilt and twist angle of the bases, and stacking interactions of the bases (Johnson and Tinoco, 1969; Moore and Wagner, 1973; Studdert and Davis, 1974), and to the unfolding of higher ordered asymmetric structures (Cheng and Mohr, 1975).

An important result which can be discerned from Table I for the complexes of DNA with $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$ at $r = 0.1$ and 0.25, and with $(\mathrm{Lys}^{77})_n(\mathrm{Leu}^{23})_m$ at $r = 0.5$ is that the leucine residues associated with the copolymers destabilize the bound fraction of DNA against thermal denaturation relative to that of $(\mathrm{Lys})_n$ bound DNA (Li et al., 1974a). Such destabilization has also been observed for the $(\mathrm{Lys}^{84.5},\mathrm{Val}^{15.5})_n$ copolymers (Mandel and Fasman, 1974), a $(\mathrm{Lys}^{50},\mathrm{Tyr}^{50})_n$ copolymer (Santella and Li, 1974), and a $(\mathrm{Lys}^{40},\mathrm{Ala}^{60})_n$ copolymer (Pinkston and Li, 1974). However, the Leu residues appear to bind and independently stabilize the melting of DNA as indicated by T_{mII}'. Thus the hydrophobic residues play a significant role, probably by interacting with the bases, in the binding of polypeptides and proteins to DNA. A similar differentiation between binding of the less basic and more basic histones to DNA in chromatin has been reported by Li et al (1973, 1975). The absorption T_{m} of the less basic fraction of the histones was found to be 72° (T_{mIII}) and at 82° (T_{mIV}) for the more basic fraction. By utilizing trypsin digests of nucleoprotein it was possible to differentiate between these two types of binding (Ansevin et al., 1971; Li et al., 1975).

(2) *The complexes which yield significantly altered CD spectra* are believed to be caused by the condensation of the DNA (Ong et al., 1976).

The absorption and CD melting profiles of such polypeptide-DNA complexes are significantly different than those discussed above. The complexes of DNA with $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$ and $(\mathrm{Lys}^{84},\mathrm{Leu}^{16})_n$, at $r = 0.5$, give CD spectra that resemble the Ψ spectra (Ong et al., 1976). As shown in Figures 5 and 9, the CD melting profiles of these two complexes are similar in shape. These curves are characterized by a large premelt, with T_{mp} at ~27°, and a very broad shoulder centered at ~32° (Figure 6). The average slope of the premelt, $[\Delta\theta_{\mathrm{p}}/\Delta T_{\mathrm{mp}}]$, which is defined by the total change in ellipticity over the entire premelting temperature range (i.e., ~25-40°), is 140 ± 20 molar ellipticity/°C for $(\mathrm{Lys}^{85})_n(\mathrm{Leu}^{15})_m$-DNA at $r = 0.5$, and 300 ± 50 molar ellipticity/°C for $(\mathrm{Lys}^{84},\mathrm{Leu}^{16})_n$-DNA at $r = 0.5$. The premelt probably results from the partial unfolding of the condensed state structure. This melt is then followed by the first melt with T_{mI} at ~50°, slightly higher than their corresponding absorption T_{mI} (see Table I). The slope of their first melts (T_{mI}) is approximately equal to the slope of their respective premelts, and may be due to the fact that melting of the free DNA is accompanied by further unfolding of the condensed structure. This is necessarily so since the melting of the unbound DNA would be characterized by a decrease in $[\theta]_{280}$ (see Figure 3), while the first melt of the condensed structure is still accompanied by an increase in $[\theta]_{280}$ (Figure 9). The T_{mII} band in CD is characterized by a very large and sharp transition, with T_{mII} slightly lower than their corresponding T_{mII} in absorption, and probably reflects the ultimate collapse of the condensed structure.

Cheng and Mohr (1974) have shown that Ψ-DNA (a condensed form of DNA) can undergo a thermal transition from the condensed structure to the B structure of DNA, and such a transition is reversible upon cooling. They have also shown that the Ψ-DNA is incapable of intercalating dyes such as ethidium bromide (Cheng and Mohr, 1975).

The condensed structures produced from the complexes studied herein do not undergo a $\Psi \rightarrow B$ transition, but achieved a total collapse in structure just prior to T_{mII}. This is expected since the Ψ-type structure produced herein arises from the binding of basic polypeptides to DNA, while the Ψ-DNA produced by polyethylene oxide-salt (PEO-salt) derives from an exclusion interaction effect resulting in a phase separation of DNA and PEO (Lerman, 1973). The Ψ-DNA in PEO solutions thus contains only DNA in a compact structure while the condensed DNA structures of the complexes contain polypeptides bound to DNA. The slightly lowered T_{mII} in CD relative to absorption for our Ψ-type DNA complex can also be understood in terms of the CD melt reflecting the thermal transition of the Ψ structure, while the absorption melt reflects only the melting of the secondary structure.

While it is true that absorption melting profiles may reflect a change in secondary structure, CD melting profiles appear to be more sensitive to higher ordered structure, the tertiary structure. This may be further supported by the fact that T_{mI} and T_{mII} from the absorption melts remain relatively unchanged for the $(Lys^{85})_n(Leu^{15})_m$-DNA complexes at different r values (Table I), while the T_{mI} and T_{mII} from the CD melting profiles of the same complex at $r = 0.5$ are quite different from that at $r = 0.1$ and 0.25. In addition, the absorption spectra of such complexes at different r values remain relatively unchanged and similar to the spectra of native DNA in solution, while the CD spectra of such a complex at $r = 0.5$ are of the Ψ structure and a structure similar to B-form DNA at $r = 0.1$ and 0.25. The T_m values for the complex at $r = 0.1$ and 0.25, obtained from CD melting profiles, are equal to those of the corresponding absorption studies. This is anticipated as both the CD and absorption melting profiles reflect only the melting of the secondary structure in this case.

The CD spectra of the $(Lys^{68},Leu^{32})_n$-DNA complexes, at $r = 0.1$ and 0.25, are nonconservative spectra of the A-like type (Ong et al., 1976). Such CD spectra are characterized by a large positive band at 270 nm and a small negative band at ~240 nm, and it has been suggested that these are also due to a condensed structure of DNA, different in asymmetry than the Ψ type (Ong et al., 1976). Here again, the absorption melting profile (Figure 11) reflects the helix-coil transition of DNA in the free and bound region, corresponding to T_{mI} and T_{mII}. It is also found that their respective absorption T_m's are independent of ratio (see Table I), while the fraction of bound DNA (A_b) is equal to the input ratio, where r_b = (Lys + Leu)/nucleotide.

The CD melting profile of such a complex at $r = 0.25$ (Figure 12) shows a very small premelt followed by an even smaller first melt and finally a very sharp transition at T_{mII} corresponding to the final melting of the condensed state DNA in the bound regions. Here again, as in the case of the Ψ structure, $(Lys^{84},Leu^{16})_n$-DNA, and $(Lys^{85})_n(Leu^{15})_m$-DNA complexes, the CD T_{mII} is slightly lower than its corresponding absorption T_m. The very broad T_{mI} band for the complex at $r = 0.1$ may be due to the simultaneous partial unfolding of the condensed structure and melting of the free DNA.

The results from the CD melting studies of the above complex are very similar to that of the H4-DNA complex (Adler et al., 1975). A CD melting profile of H4-DNA also shows a significant premelt, as does both the C-fragment H4-DNA and N-fragment H4-DNA melting profiles (H4 cyanogen bromide cleavage products). This is not surprising since the CD of the H4-DNA complex also resembles that of the A-like spectra, and may result from the same type of condensed structure as the complex of DNA with $(Lys^{68},Leu^{32})_n$.

Chromatin Structure. The relevance of the premelt of polypeptide-DNA complexes to chromatin structure may be viewed as follows. The Ψ-type structure of these complexes is comparable to the Ψ-DNA (Jordan et al., 1972). Such CD spectra arise from a condensed structure of DNA. The DNA in nuclei and in chromatin is known to be in a highly condensed state. The CD spectra of chromatin have been shown to be altered and diminished, $[\theta]_{275}$, relative to DNA (Permogorov et al., 1970; Simpson and Sober, 1970; Shih and Fasman, 1970; Wilhelm et al., 1970; Henson and Walker, 1970) and the CD spectra of nuclei were found to be similar but significantly different than that of chromatin (Wagner and Spelsberg, 1971). If it is assumed that the condensation of DNA in chromatin, brought about by specific histone-histone interaction, causes this altered CD spectra, (a less structured form than Ψ-DNA) and is not due to a transition to a C-form DNA, then the premelt behavior of nucleoprotein can be explained as follows. In the total nucleoprotein complex no premelt is found; however, on removing the non-histones it is restored. Therefore, the non-histones are preventing the unraveling of the condensed state. Removal of H2A and H2B or H1 also restores the premelt, therefore interactions of these histones with others prevent opening of the condensed state. It is also known from hydrodynamic studies that chromatin contains DNA in a collapsed non-flexible structure (Henson and Walker, 1971) and that high salt, which removes histones, allows the DNA to become flexible, simultaneously yielding a CD spectra similar to native DNA. Viscosity studies have shown that urea opens up the condensed form of DNA in chromatin (Henson and Walker, 1971; Bartley and Chalkley, 1973), lowers the melting temperature of nucleoprotein (Chang and Li, 1974), and causes an increase in the positive CD band of chromatin (Chang and Li, 1974; Bartley and Chalkley, 1968, 1973). Thus by destroying or weakening histone-histone interactions the condensed form of chromatin is relaxed.

The above implies that the small premelt of native B-DNA is due to unravelling of a small amount of condensed structure. The Raman spectra show that the B-form geometry of DNA remains up to the melting point (Erfurth and Peticolas, 1975). Viscosity studies have demonstrated that on lowering the ionic strength the viscosity of DNA increases, which would support this hypothesis (Ross and Schruggs, 1968, and references cited herein). Thus in chromatin and in the nucleus a part of the DNA is folded into a condensed state, a highly asymmetric folded structure, comparable to Ψ-DNA. This is probably the main cause of the altered CD spectra of chromatin, rather than the previous suggestions of the C-form DNA.

Circular Dichroism Melting Profile of Chromatin. The following structural proposal is offered as a tentative explanation to account for the multistep CD melting profile of chromatin. The condensed state of DNA in chromatin is depicted as I in Figure 14. The DNA is composed of two distinct repeating regions; Ia, unbound DNA, folded in a compact manner (perhaps similar to Ψ-DNA; unbound-DNA may also include weakly bound polypeptides) and Ib, polypeptide-bound DNA in folded regions. This structure gives rise to a higher order asymmetry than observed in native DNA. Upon heating the premelt stage, $T_{\rm mp}$, is first observed, in which the unbound folded DNA regions unravel, while the polypeptide-bound DNA remains unchanged, resulting in the condensed structure II, slightly different from the original structure I. In structure II the unbound DNA is unfolded (although still native B form), but the total structure still maintains a higher order asymmetry. The asymmetry of structure II could be attributed to either II(a) a local asymmetry due to polypeptide-bound DNA regions or II(b) the asymmetry of the total structure including unbound and bound DNA. On raising the temperature further, the first melt, $T_{\rm mI}$, is observed. This is due either to the melting of the unbound DNA and a partial unfolding of regions in IIa, resulting in structure III, where the local asymmetry, IIIa, is partially unfolded, relative to IIa or to structure IV if the asymmetry in II is due to structure IIb. The asymmetry in IV would then be different from IIb, since the unbound regions are melted. The final melt, $T_{\rm mII}$, is due to the melting of polypeptide-bound DNA regions yielding structure V. This melt can be multiphasic due to the binding of several proteins (e.g., histones) with different binding constants. The final melt produces the total collapse of the condensed structure to produce denatured single-strand DNA, V. It is not known whether the polypeptides are still bound to the denatured DNA.

The Modification of Chromatin Structure. The alterations of the properties of chromatin due to histone modification can be viewed in this light. The degree of acetylation of histones in lymphocytes increased in the first 15 min after exposure to mitogens, such as phytohemagglutinin (Pogo et al., 1966). This change is accompanied by changes in accessibility of the DNA to dyes such as acridine orange (Killander and Rigler, 1965a,b). Studies by Adler et al. (1974) on the association of acetylated H4 with DNA showed that the CD spectra of the complex were similar to native DNA, having reversed the condensation caused by unacetylated H4. Similarly it has been shown that phosphorylation of H1 also causes a reversal of the altered CD spectra of the complex, H1-DNA, yielding a native like structure (Adler et al., 1971, 1972). Both these CD changes indicate that these enzymatic modifications cause an opening of the condensed nucleoprotein. This interpretation agrees with that of Bradbury (1975) who states that phosphorylated H1 is the mitotic trigger and controls the aggregation of a central interacting region of the H1 to molecules with a corresponding region of another H1 elsewhere on the chromosome, thus promoting the coiling and condensation characteristic of the metaphase chromosome. Marks et al. (1973) also relate the phosphorylation of H1 to the separation and condensation of the chromatides before and during mitosis. Barserga and coworkers (Lin et al., 1974) have demonstrated CD changes of chromatin of cells stimulated to proliferate. Thus the condensation of DNA in chromatin is controlled by histone-histone interactions and these specific interactions can be modified by enzymatic modification.

The thermal denaturation studies of polypeptide–DNA interactions have opened a new approach to study the condensation of DNA in many biological systems; chromatin, bacteriophage, viruses, etc., and to probe the specificity of protein–DNA interactions in the above systems as well as others, e.g., unwinding proteins, lac repressor, etc.

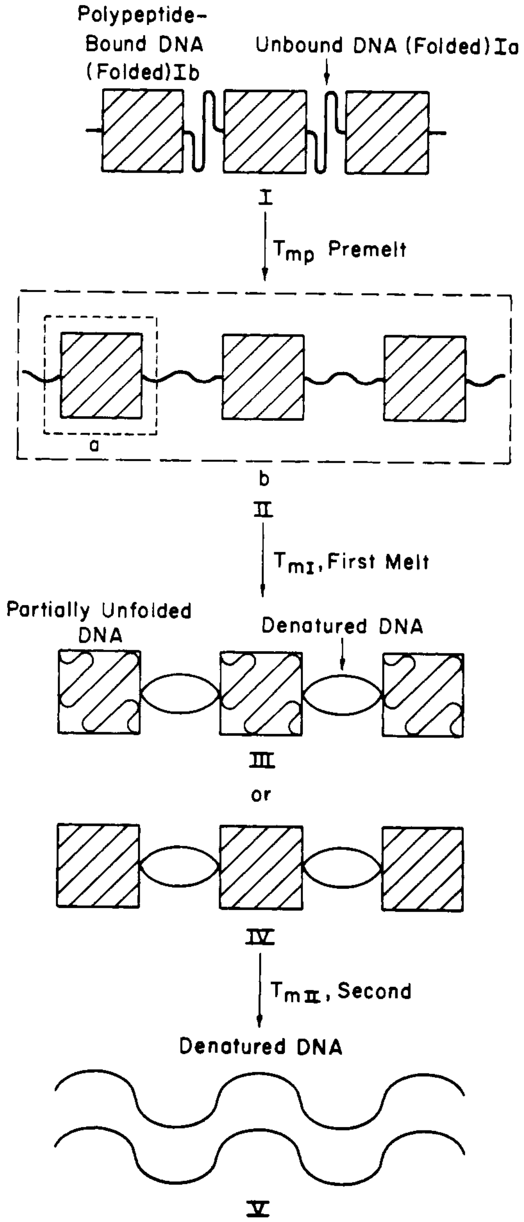

FIGURE 14: Schematic diagram of proposed multistep temperature melt out of chromatin. I, native chromatin; II, chromatin after premelt, $T_{\rm mp}$, IIa, local symmetry, IIb, overall symmetry; III, chromatin after first melt out, $T_{\rm mI}$, where native unbound DNA is melted, as well as change in bound DNA structure; IV, alternate structure after first melt out, $T_{\rm mI}$ where only unbound DNA has melted; V, denatured chromatin after second melt out, $T_{\rm mII}$.

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