

# Chromatin Models. Interactions between DNA and Polypeptides Containing L-Lysine and L-Valine: Circular Dichroism and Thermal Denaturation Studies<sup>†</sup>

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**ABSTRACT:** The interaction of calf thymus DNA with statistical copolymers of L-lysine and L-valine [poly(L-Lys<sup>100f</sup>-L-Val<sup>f</sup>)] and block copolymers [poly(L-Lys)<sup>100f</sup>-poly(L-Val)<sup>f</sup>] were investigated as a function of ionic strength using circular dichroism (CD) spectroscopy. It was found that valine suppresses the ability of the copolymer-DNA complexes to yield a  $\Psi$ -type CD spectra as found for poly(L-Lys)-DNA [Jordan, C. F., Lerman, L. S., and Venable, J. N. (1972), *Nature (London)*, *New Biol.* 236, 67] and lowers the ionic strength at which CD distortion occurs. Thermal denaturation, simultaneously monitoring 280-nm ellipticity,  $[\theta]_{280}$ , and hyperchromicity,  $h_{280}$ , was carried out on annealed complexes of poly(L-Lys)-DNA, poly(L-Lys<sup>84.5</sup>-L-Val<sup>15.5</sup>)-DNA, poly(L-Lys)<sup>87.2</sup>-poly(L-Val)<sup>12.8</sup>-DNA and directly mixed complexes of poly(L-Lys)-DNA, in  $2.5 \times 10^{-4}$  M EDTA, pH 7.0 solution. The CD denaturation of uncomplexed DNA at several ionic strengths was also determined to examine pre-melting. Despite the inability of both statistical and block co-

polymers of L-Lys and L-Val to form  $\Psi$ -type complexes with DNA, they bind as well to DNA as does poly(L-Lys) and give rise to a thermal denaturation pattern showing bound peaks between 90 and 100 °C, seen clearly with CD denaturation. The thermal denaturation of mixed and annealed complexes of poly(L-Lys)-DNA shows similar patterns in hyperchromicity changes as a function of temperature but very different CD melts. From the CD melt of annealed poly(L-Lys)-DNA, it appears that aggregation and long-range order of the complex are significant in low salt ( $2.5 \times 10^{-4}$  M EDTA) as well as in 1.0 M NaCl. These studies further illustrate the importance of the nature of nonionic interactions (hydrophobic) between polypeptides and DNA in determining the behavior of their complexes, such as causing condensation into higher order asymmetric structures. In light of these observations, the possible significance to the CD melting of chromatin and the validity of identification of C-form DNA by CD spectroscopy are discussed.

Complexes between poly(L-Lys)<sup>1</sup> and DNA (L-DNA) have been widely used as a model for the interaction of histones with DNA (Olins et al., 1967; Shih and Bonner, 1970) and as a probe of the structure of chromatin (Itzhaki, 1971; Li, 1972; Clark and Felsenfeld, 1971; Umiel and Plaut, 1973). More recently, the interaction between DNA and random copolymers containing other amino acids in addition to Lys, i.e., Leu (Ong et al., 1976; Ong and Fasman, 1976), Ala (Sponar et al., 1973), as well as sequential copolymers containing L-Lys, L-Ala, L-Pro, L-Gly (Sponar et al., 1973, 1974), L-Lys, L-Gly (Brown et al., 1974), and L-Lys and L-Tyr (Santella and Li, 1974) have been studied as a means of understanding the role of peptide sequence and secondary structure on the strength, specificity, and mode of binding with DNA.

It has been observed that poly(L-Lys) forms qualitatively different complexes with DNA, depending on the salt con-

centration at which binding occurs. When the complex is prepared by direct mixing at salt concentrations on the order of  $1.0 \times 10^{-3}$  M, it is found that: the circular dichroism (CD) spectrum of the complex depends linearly on the ratio of Lys to DNA; the CD spectrum of Lys bound regions in the complex is similar to that of DNA in 6 M LiCl (Chang et al., 1973). On the other hand when poly(L-Lys) is bound at NaCl concentrations of around 1.0 M, the resulting complex shows cooperative binding characterized by a very intense CD spectrum with a peak ellipticity of  $-100,000$  at 270 nm (Shapiro et al., 1969), CD changes which are nonlinear with respect to Lys input, and significant light scattering at wavelengths greater than 320 nm (Carroll, 1972). Similar optical properties have also been seen in complexes between H1 histone and DNA dialyzed down to 0.15 M NaCl (Fasman et al., 1970; Sponar and Fric, 1972), films of Li-DNA and Li-d(A-T) at 92% or lower relative humidity (Brunner and Maestre, 1974) and films of Na-DNA at 75% humidity (Tunis-Schneider and Maestre, 1970) and DNA in solutions of NaCl and polyethylene oxide (the  $\Psi$  form) (Jordan et al., 1972).

Following verification that the three predominant canonical forms of DNA, A, B, and C, as determined by x-ray crystallography, have three distinct CD spectra (Tunis-Schneider and Maestre, 1970), CD spectroscopy has been utilized to study conformational changes in DNA: in salt solutions (Ivanov et al., 1973; Zimmer and Luck, 1973), as a function of temperature prior to melting (Gennis and Cantor, 1972; Studdert et al., 1972), in complexes of polypeptides with DNA (Chang et al., 1973), in complexes of histones with DNA (Fasman et al., 1970; Shih and Fasman, 1971) and in chromatin (Hanlon et al., 1972; Shih and Fasman, 1970). This paper examines the optical properties of gradient dialyzed complexes between

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<sup>1</sup> Abbreviations used: CD, circular dichroism; L-DNA, poly(L-lysine)-DNA; block LV-DNA, poly(L-lysine)<sup>87.2</sup>-poly(L-valine)<sup>12.8</sup>-DNA complex; statistical LV-DNA, poly(L-lysine<sup>84.5</sup>-L-valine<sup>15.5</sup>)-DNA complex;  $T_{mf}$ , maximum of derivative of hyperchromicity with respect to temperature of unbound segments of DNA;  $T_{m1}$ , maximum of derivative of hyperchromicity with respect to temperature of bound segments of DNA;  $T_{m2}$ , maximum of derivative of hyperchromicity with respect to temperature of bound segments of DNA; EDTA, ethylenediaminetetraacetic acid; Na<sub>2</sub>EDTA, disodium ethylenediaminetetraacetate; Cbz, benzyloxycarbonyl; dp, degree of polymerization.

TABLE I: Copolymers of L-Lys and L-Val. Polymerization Conditions, Amino Acid Ratios, and Molecular Weights.

Polymer	Lys:Val <sup>a</sup> Ratio	A/I <sup>b</sup>	$[\eta]^g$	Mol Wt <sup>d</sup>	dp
GF-21-353-25	100.0:0.0	200	0.81	53 000	322
GF-21-329-23	100.0:0.0	200	1.48	106 000	645
GF-23-48-17	94.4:5.6	25	2.98	216 000	1340
GF-23-44-24	86.8:13.2	25	1.16	80 000	512
GF-23-142-25	85.5:15.5	25	<sup>c</sup>	147 000 <sup>c</sup>	950
GF-23-448-15 <sup>e</sup>	87.2:12.8	20 <sup>f</sup>		124 000 <sup>c</sup>	756

<sup>a</sup> Determined by amino acid analysis. <sup>b</sup> Anhydride to initiator ratio. <sup>c</sup> Not soluble enough for viscosity measurement. Molecular weight estimated from urea gel electrophoresis (Walter and Fasman, to be published). <sup>d</sup> Estimated from calibration curve for poly-L-lysine (Applequist, 1959). <sup>e</sup> Block copolymer, poly(L-Lys)<sup>87.2</sup>-poly(L-Val)<sup>12.8</sup>. <sup>f</sup> *n*-Hexylamine initiator for the *N*<sup>ε</sup>-Cbz-L-lysine-*N*-carboxyanhydride. <sup>g</sup> 1 M NaCl pH 4.0.

random and block copolymers containing Lys and Val [poly(L-Lys)<sup>100f</sup>-poly(L-Val)<sup>f</sup>] and calf thymus DNA as a function of ionic strength and compares these results with those of gradient dialyzed poly(L-Lys)-DNA (L-DNA) complexes at the same lysyl to phosphate ratios (*r*).

Thermal denaturation is a useful tool to determine the extent and strength of binding of DNA to polypeptides (Li, 1973) and histones (Ansevin and Brown, 1971) and as a probe of nucleohistone structure (Subirana, 1973). This method has been extended in a previous paper (Mandel and Fasman, 1974) by simultaneous measurement, at a single wavelength, of both absorption and CD changes prior to and during melting. At temperatures below melting, the ellipticity at 280 nm of DNA is very sensitive to temperature (known as premelting) (Gennis and Cantor, 1972), and is thought to reflect conformational changes in the DNA double helix structure. In addition, ellipticity changes prior to and during thermal denaturation have been followed in complexes of H4 and DNA (Adler et al., 1975), and in chromatin (Henson and Walker, 1970; Wilhelm et al., 1974).

To better understand the significance of premelting, the thermal denaturation of DNA, monitored by simultaneous absorption and CD measurement, was carried out over a wide range of salt concentration. In addition this method has been applied to both directly mixed and annealed complexes of poly(L-Lys) with DNA as a probe of structural differences between these two distinct complexes and as models for the understanding of the significance of the premelting phenomena observed in other polypeptide and protein complexes with DNA, as well as in chromatin. Furthermore, the thermal denaturation of annealed complexes between the statistical copolymer poly(L-Lys)<sup>84.5</sup>-L-Val<sup>15.5</sup> and the block copolymer poly(L-Lys)<sup>87.2</sup>-poly(L-Val)<sup>12.8</sup> with DNA has been studied to examine the role that hydrophobic residues play in interactions between proteins and DNA and their effect on the structure and physical properties of the complex.

#### Experimental Section

*Calf thymus DNA* was prepared as previously described (Fasman et al., 1970) and had a molecular weight of approximately  $10-12 \times 10^6$  as determined by ultracentrifuge sedimentation.

*Poly-L-lysine* was prepared as previously described (Fasman et al., 1961) with intrinsic viscosity and estimated molecular weight shown in Table I.

*Random copolymers of L-lysine and L-valine* [poly(L-lysine)<sup>100f</sup>-L-valine<sup>f</sup>] were synthesized as previously described (Mandel and Fasman, 1975).

*Block copolymer, (L-Lys)<sup>87.2</sup><sub>n</sub>(L-Leu)<sup>12.8</sup><sub>m</sub> Synthesis.* *N*<sup>ε</sup>-Cbz-L-lysine-*N*-carboxyanhydride (0.603 g) (Fasman et al., 1961) was dissolved in dry dioxane (61 ml) and 0.328 ml of 0.300 *N*-hexylamine, in benzene, was added to initiate polymerization (anhydride/initiator = 20). The solution became viscous after standing 5 days. L-Valine-*N*-carboxyanhydride (0.0497 g) (Kubota and Fasman, 1975), dissolved in freshly distilled benzene (5 ml), was added to the above viscous solution which was stirred for 5 min and allowed to stand for 4 days. The viscous solution was diluted with CHCl<sub>3</sub> (60 ml), stirred well, and treated with HCl gas and HBr gas (30 min each) in the manner described by Fasman et al. (1961). After stirring for 2 h, the white precipitate which had formed was allowed to settle and the supernatant was removed by suction. The precipitate was dissolved in H<sub>2</sub>O (300 ml), the solution adjusted to pH 7 with 1 *N* HCl and extracted twice with ether, the pH adjusted to 5.0, and the solution dialyzed in a fiber dialyzer (Bio-Rad, mini plant No. c/HFD-15, molecular weight cutoff 5000) for 4 h vs. H<sub>2</sub>O, 4 h vs. 0.01 *N* HCl, and finally lyophilized (weight, 0.15 g).

The amino acid compositions as well as intrinsic viscosities and estimated molecular weights are shown in Table I.

*Annealed complexes* were prepared by addition of a 2 M NaCl solution of polypeptide to a 2 M NaCl solution of DNA to obtain the correct lysyl to phosphate ratio, with subsequent dialysis at 4 °C from 1.5 M NaCl to the required salt concentration. Both linear gradient and exponential gradient dialysis were used (Carroll, 1971). Exponential gradient dialysis was performed at a rate such that the complexes went from 1.5 to 0.5 M salt over the course of at least 5 h.

Dialysis bags were prepared by boiling in 5% NaHCO<sub>3</sub>-2 × 10<sup>-3</sup> M Na<sub>2</sub>EDTA, washing in double distilled water, and boiling with double-distilled water prior to use.

The concentration of DNA stock solutions was determined by absorption measurement with  $\epsilon_{260}$  6800. Concentration of poly(L-Lys) stock solutions was determined by biuret determination (Zamenhof, 1957) and the L-Lys, L-Val copolymer concentrations by micro-Kjeldahl analysis (Lang, 1958). Due to volume changes during dialysis it was necessary to determine the DNA concentration in the complex. This was determined from OD<sub>max</sub> of 0.5 *N* HClO<sub>4</sub> hydrolysates (30 min in boiling water bath) (Adler et al., 1971) or by 1:1 dilution of the complex with 4.0 M NaCl. Both methods yield consistent results.

*Ultraviolet absorption* spectra were measured in a Cary 14 recording spectrophotometer at 23 °C.

*Circular dichroism* spectra were measured in a Cary 60 recording spectropolarimeter with a Model 6001 CD attach-

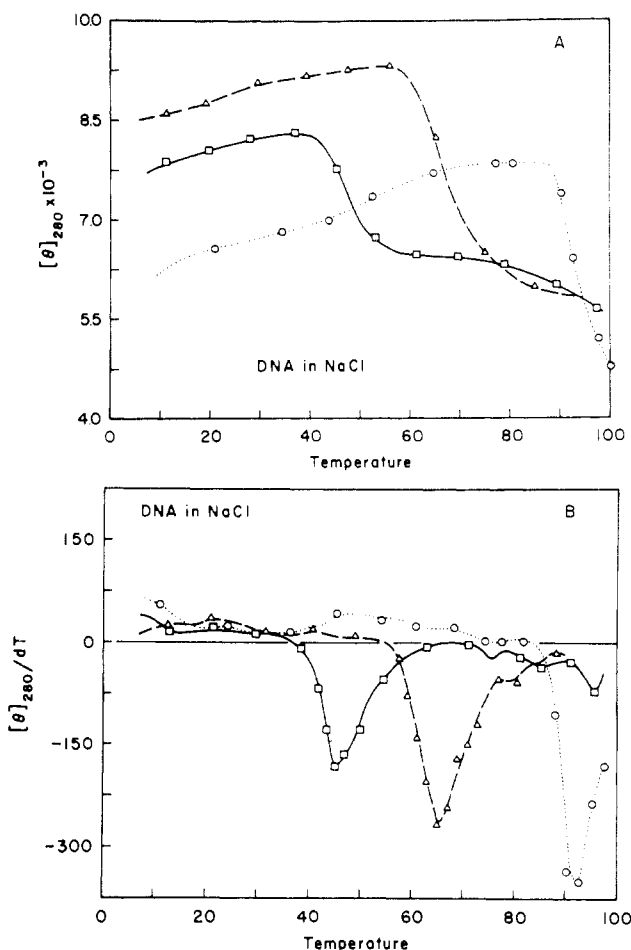


FIGURE 1: Denaturation of DNA in  $2.5 \times 10^{-4}$  M EDTA, pH 7.0 (□-□); 0.012 M NaCl +  $2.5 \times 10^{-4}$  M EDTA, pH 7.0 (Δ-Δ); 1.0 M NaCl +  $2.5 \times 10^{-4}$  M EDTA, pH 7.0 (○·····○). (A) Ellipticity,  $[\theta]_{280}$ , vs. temperature. (B) Derivative of ellipticity,  $[\theta]_{280}/dT$ , with respect to temperature.

ment as previously described (Adler et al., 1971). Ellipticity,  $[\theta]$ , is expressed in units of  $\text{deg mol}^{-1} \text{cm}^{-1}$ .

**Thermal denaturation** measurements were carried out as previously described (Mandel and Fasman, 1974) on solutions with DNA concentrations of  $1.0\text{--}2.3 \times 10^{-4}$  M phosphate.

To facilitate comparison of different complexes which scatter light and therefore have significantly different extinction coefficients, the absorption denaturation curves are normalized and plotted as hyperchromicity derivatives vs. temperature

$$\left(\frac{dh}{dT}\right)_i = \frac{1}{\epsilon_0} \left( \frac{\epsilon_{i+1} - \epsilon_{i-1}}{T_{i+1} - T_{i-1}} \right)$$

where  $(dh/dT)_i$  is the derivative of hyperchromicity with respect to temperature at temperature corresponding to point  $i$ ,  $i \pm 1$  refers to data points adjacent to  $i$ , and  $\epsilon_0$  is the lowest measured extinction. CD denaturation curves are drawn as ellipticity,  $[\theta]_{280}$ . The derivative of ellipticity with respect to temperature  $d[\theta]_{280}/dT$  vs. temperature is useful for identification of thermal transitions and measurement of the premelting slope. The premelting slope is defined as the average value of  $d[\theta]_{280}/dT$  between approximately 5 and 35 °C.

Direct mixed complexes were prepared by slowly adding an equal volume of polypeptide at the correct concentration in  $2.5 \times 10^{-4}$  M EDTA (pH 7.0) in a solution of DNA in  $2.5 \times 10^{-4}$  M EDTA and dialyzing the resulting complex in a large volume of  $2.5 \times 10^{-4}$  M EDTA (pH 7.0).

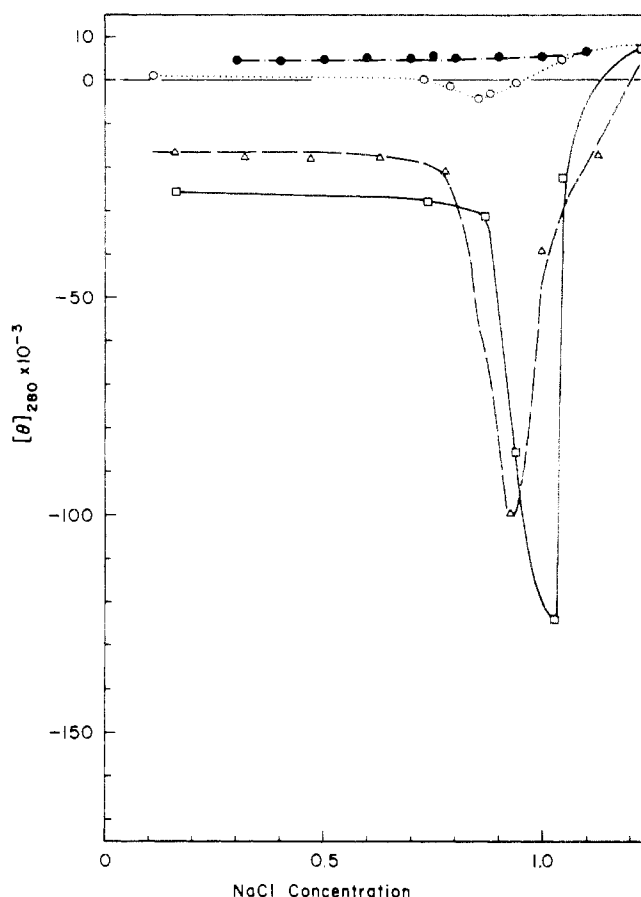


FIGURE 2: Ellipticity,  $[\theta]_{280}$ , vs. NaCl concentration of polypeptide-DNA complexes, at  $r = 0.5$ . Poly(L-lysine)-DNA (□-□); poly(L-lysine<sup>94,4</sup>-L-valine<sup>5,6</sup>)-DNA (Δ-Δ); poly(L-lysine<sup>86,8</sup>-L-valine<sup>13,2</sup>)-DNA (○·····○); poly(L-lysine<sup>87,2</sup>-poly(L-valine)<sup>12,8</sup>-DNA (●····●).

TABLE II: Thermal Denaturation of DNA vs. Salt Concentration as Measured by Ellipticity  $[\theta]_{280}$  and Hyperchromicity at 280 nm.

NaCl	$T_m(\text{absorption})$ $\pm 1.5^\circ \text{C}$	$T_m(\text{ellipticity})$ $\pm 1.5^\circ \text{C}$	$\Delta v$ Premelt Slope <sup>b</sup>	$[\theta]_{280}$
0.0007 <sup>a</sup>	47.3	47.8	$24.0 \pm 8$	7900
0.0120	65.2	65.7	$19.0 \pm 8$	9300
1.00	91.6	91.9	$25.4 \pm 8$	7900

<sup>a</sup>  $\text{Na}^+$  concentration corresponding to  $2.5 \times 10^{-4}$  M EDTA, pH 7.0. <sup>b</sup> The average value of premelt was determined by averaging the values of  $(d[\theta]_{280}/dT)_i$  for all measured points between approximately 5 and 35 °C.

## Results

**Premelting of DNA.** In order to examine the salt dependence of premelting, thermal denaturation of DNA monitored at 280 nm was carried out in  $2.5 \times 10^{-4}$  M EDTA and 0.012 M and 1.0 M NaCl and is shown in Figure 1. The calculated average slope of the premelt, the highest attained ellipticity, and the  $T_m$  from both absorption (not shown) and CD are summarized in Table II. It can be clearly seen from the parallel lines at low temperature in Figure 1A that the slope of the premelt is independent of salt over a wide range of salt concentration. This is in general agreement with previous results obtained at 0.5 and 0.05 M NaCl (Gennis and Cantor, 1972). It should also be noted that the maximum attained ellipticity, as well as the

TABLE III: Critical NaCl Concentration for Maximal Circular Dichroism Changes in Copolymer-DNA Complex.

Copolymer	$r$	Critical [NaCl]	$[\theta]_{280}$
Lys	0.5	1.02	-125 000
Poly(L-Lys <sup>94.4</sup> -L-Val <sup>5.6</sup> )	0.5	0.93	-100 000
Poly(L-Lys <sup>86.8</sup> -L-Val <sup>13.2</sup> )	0.5	0.83	-4 000
Poly(L-Lys <sup>87.2</sup> -poly(L-Val) <sup>12.8</sup> )	0.5	<0.10	4 500

ellipticity at constant temperature below melting, are not monotonically decreasing with salt. The peak ellipticity of DNA in 0.012 M NaCl is higher than in either  $2.5 \times 10^{-4}$  M EDTA (pH 7.0) or 1.0 M NaCl. At the same time the temperature of melting increases and the half-width or cooperativity of melting decreases with increasing salt (Figure 1B).

**Salt Dependence of the Circular Dichroism of Various Polypeptide-DNA Complexes.** Linear gradient annealed complexes of poly(L-Lys), poly(L-Lys<sup>94.4</sup>-L-Val<sup>5.6</sup>), poly(L-Lys<sup>86.8</sup>-L-Val<sup>13.2</sup>), and poly(L-Lys<sup>87.2</sup>-poly(L-Val)<sup>12.8</sup> with DNA, having lysyl to phosphate ratios of  $r = 0.50$ , were prepared at various salt concentrations. The ellipticity at 280 nm,  $[\theta]_{280}$ , vs. NaCl is plotted in Figure 2. Poly(L-Lys) shows a very sharp increase in the ellipticity magnitude at  $\sim 1.03$  M salt (termed the critical salt concentration) to yield  $[\theta]_{270} = -128\ 000$ . At lower ionic strengths,  $[\theta]_{280}$  rapidly approaches  $-25\ 000$  between 0.1 and 0.5 M NaCl and further increases to  $\sim -10\ 000$  at  $2.5 \times 10^{-4}$  M EDTA (not shown). This behavior is in agreement with former studies (Carroll, 1972) but in the experiments herein the maximum change occurs at higher salt, probably due to the higher molecular weight poly(L-Lys) used herein relative to the former study ( $dp \sim 75$ ); also, the ellipticity in the former study drops off less rapidly at low salt than in this current report. Other studies have seen large CD changes at 1.0 M NaCl (Haynes et al., 1970; Shapiro et al., 1969).

Poly(L-Lys<sup>94.4</sup>-L-Val<sup>5.6</sup>)-DNA complexes show a similar salt dependence to poly(L-Lys)-DNA but the large ellipticity change occurs at lower salt (0.93 M) for poly(L-Lys<sup>94.4</sup>-L-Val<sup>5.6</sup>)-DNA. In complexes of poly(L-Lys<sup>86.8</sup>-L-Val<sup>13.2</sup>)-DNA, the maximal  $[\theta]_{280}$  decrease occurs at a still lower salt concentration with a rather dramatic suppression of the large negative CD band (Figure 2). This complex reaches its maximum negative ellipticity at 0.85 M NaCl with a small negative  $[\theta]_{280}$  (-4500). The DNA positive band centered at 280 nm quickly returns as the salt concentration decreases below 0.73 M and, in  $2.5 \times 10^{-4}$  M EDTA, the complexes show a CD spectrum similar to that of DNA alone (not shown). The CD of complexes between the block copolymer poly(L-Lys<sup>87.2</sup>-poly(L-Val)<sup>12.8</sup> and DNA show even less of a decrease in  $[\theta]_{280}$  than that of the comparable statistical copolymer. The critical salt dependence (concentration for maximal CD change) of the CD of these complexes is summarized in Table III.

The CD spectrum of poly(L-Lys<sup>84.5</sup>-L-Val<sup>15.5</sup>)-DNA (statistical LV-DNA) as a function of  $r$  at 0.78 M NaCl was also studied (not shown). The ability of this polypeptide to distort the CD of DNA is highly suppressed compared with poly(L-Lys), and the CD of statistical LV-DNA with  $r = 0.7$  in 0.78 M NaCl has the appearance of "C-form DNA". The CD spectra of L-DNA and LV-DNA complexes ( $r = 0.125$  and 0.5), in  $2.5 \times 10^{-4}$  M EDTA (pH 7.0 at room temperature), are shown in Figure 3. At  $r = 0.125$  the CD spectrum of the LV-DNA complex is identical with that of DNA alone,

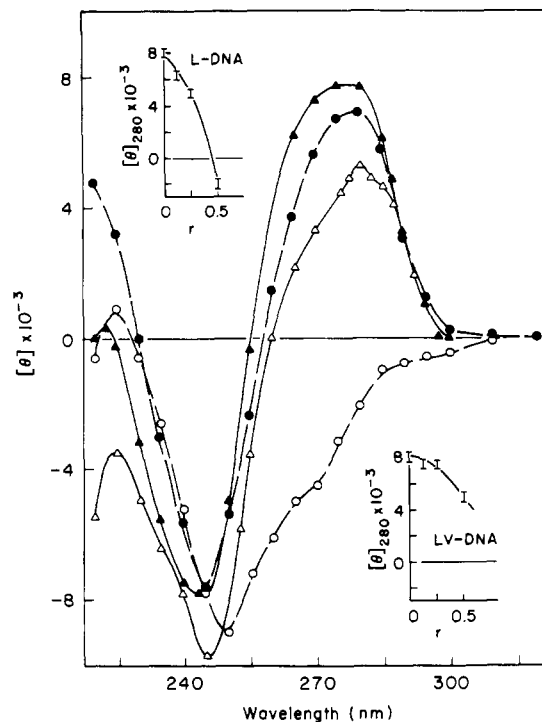


FIGURE 3: Circular dichroism spectra of poly(L-Lys)-DNA (L-DNA) and poly(L-Lys<sup>84.5</sup>-L-Val<sup>15.5</sup>)-DNA (LV-DNA) at various  $r$  values in  $2.5 \times 10^{-4}$  M EDTA, pH 7.0. L-DNA:  $r = 0.125$  (●-●);  $r = 0.50$  (○-○). LV-DNA:  $r = 0.125$  (▲-▲);  $r = 0.5$  (△-△).

while the L-DNA complex has a slightly depressed  $[\theta]_{280}$ . At  $r = 0.5$  the CD spectrum of the LV-DNA complex is significantly altered, while the L-DNA spectrum has totally lost the positive  $[\theta]_{280}$  band. The insets in Figure 3 show the relative change of  $[\theta]_{280}$  vs.  $r$  for both complexes, showing the greater effect of poly(L-Lys).

**Thermal Denaturation of Complexes.** Complexes of directly mixed poly(L-Lys)-DNA show two distinct thermal transitions as monitored by the hyperchromicity at 280 nm,  $h_{280}$ , and plotted as the derivative  $dh/dT$  (Figure 4A). The first transition corresponding to free DNA occurs at an average temperature,  $T_{mf} = 47^\circ\text{C}$  for  $r = 0.25$  to  $r = 0.50$ . At  $r = 0.5$  the melting of bound segments appears to comprise two separate transitions, the first,  $T_{m1} = 92 \pm 2^\circ\text{C}$ , and the second,  $T_{m2} = 97 \pm 2^\circ\text{C}$ . The ratios of bound to total DNA are obtained from the area under the melting of bound segments divided by the total area under the hyperchromicity curve (Li, 1973). The parameters characteristic of the hyperchromic melting are summarized in Table IV.

The  $[\theta]_{280}$  thermal denaturation for these directly mixed complexes is seen in Figure 4B and is summarized in Table IV. These curves show additional changes superimposed on the thermal denaturation peaks of bound and free regions. This makes interpretation more difficult though potentially more interesting. The 280-nm premelt slope,  $[\theta]_{280}/dT$ , for the mixed complexes of L-DNA is approximately the same as that of DNA. The transition corresponding to free DNA ( $T_{mf}$ ) becomes smaller and more obscured by the background premelt. In the transition region of bound DNA, the  $[\theta]_{280}$  changes are more complex than for the hyperchromicity curves and clearly demonstrate the presence of at least two separate bands, a small increase at  $T_{m1} = 88^\circ\text{C}$  followed by a large decrease at  $T_{m2} = 95^\circ\text{C}$ . These temperatures agree well with those determined by hyperchromicity measurements. They are

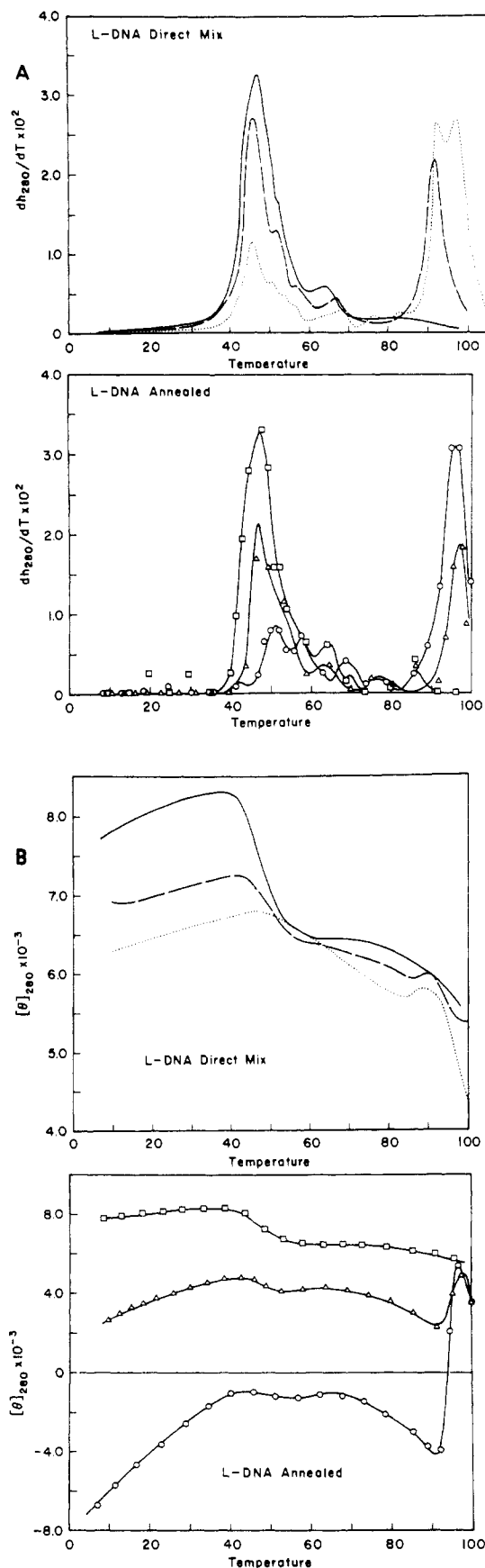


FIGURE 4: Thermal denaturation of directly mixed and annealed complexes of poly(L-Lys)-DNA (L-DNA) at several  $r$  ratios. Directly mixed,  $r = 0.0$  (—);  $r = 0.25$  (---);  $r = 0.50$  (···). Annealed complexes,  $r = 0.0$  (□-□);  $r = 0.30$  (Δ-Δ);  $r = 0.6$  (O-O). (A) Derivative of hyperchromicity with respect to temperature,  $dh_{280}/dT$ , vs. temperature. (B) Ellipticity,  $[\theta]_{280}$ , vs. temperature.

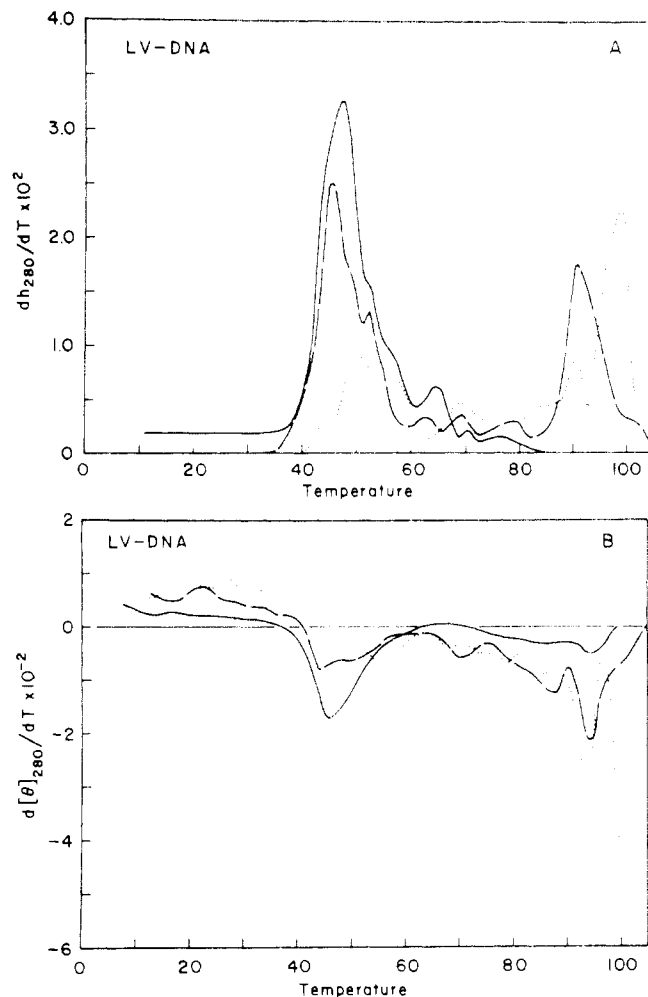


FIGURE 5: Thermal denaturation of annealed complexes of statistical poly(L-Lys<sup>84.5</sup>-L-Val<sup>15.5</sup>)-DNA at various  $r$  values.  $r = 0.0$  (—);  $r = 0.25$  (---);  $r = 0.50$  (···). (A) Derivative of hyperchromicity with respect to temperature,  $dh_{280}/dT$ , vs. temperature. (B) Derivative of ellipticity with respect to temperature,  $d[\theta]_{280}/dT$ , vs. temperature.

several degrees lower than previous reported results at  $T_{m2} = 99.0^\circ\text{C}$  (Li et al., 1974a).

Complexes of annealed poly(L-Lys)-DNA (L-DNA) show approximately the same hyperchromicity curves (Figures 4A) as for the direct mixed complexes. The average melting temperature of L-Lys-bound DNA with  $r = 0.25$  to  $r = 0.50$  is  $T_{m2} = 97^\circ\text{C}$ . This value is also several degrees lower than previous results (Li et al., 1974a) of  $T_{m2} = 104^\circ\text{C}$ . The melting of the free DNA in annealed complexes with  $r = 0.5$  occurs at an elevated temperature ( $T_{mf} = 51^\circ\text{C}$ ) relative to free DNA.

The CD denaturation of the annealed L-DNA complexes, as seen in Figure 4B and summarized in Table IV, are very different from those of the directly mixed complexes. The average temperature of melting of the bound segments is  $T_{m2} = 96.0^\circ\text{C}$ , slightly lower than  $T_{m2}$  as determined from the hyperchromicity change. The magnitude of the second transition,  $T_{m2}$ , monitored by CD is very large, positive, and nonlinear with respect to the fraction of DNA covered. In addition, a decrease in the CD occurs at temperatures higher than this transition, i.e., above  $98^\circ\text{C}$ . The premelt slope of the annealed complexes is much larger than that of DNA and increases with increasing content of lysine. The CD melt of the unbound DNA at higher  $r$  ratios is obscured due to the premelting changes and the decreased magnitude of the ellipticity change on melting.

TABLE IV: Thermal Denaturation of Complexes of DNA and Poly(L-Lys) and Poly(L-Lys<sup>84.5</sup>-L-Val<sup>15.5</sup>).

Polypeptide	Type of Complex	Nominal Input Ratio $r$	Calcd Ratio $r^a$	$d[\Theta]_{280}/dT$ Premelt	$T_{mf}^b$	Hyperchromicity		Ellipticity	
						$T_{m1}$	$T_{m2}$	$T_{m1}$	$T_{m2}$
Poly(L-Lys)	Direct mix	25	0.28	20	48	91.5		88.2	93.5
		50	0.46	20	47	92.0	97.0	88.2	97.0
Poly(L-Lys)	Annealed	30	0.34	100	47	96.3		95.0	100
		60	0.60	180	51	97.2		94.5	100
Poly(L-Lys <sup>84.5</sup> -L-Val <sup>15.5</sup> )	Annealed	25	0.32	48	46	90.6		94.3	
		50	0.52	70	50	90.6	98.2	93.7	100
Poly(L-Lys) <sup>87.2</sup> -poly(L-Val) <sup>12.8</sup>	Annealed	25	0.31	45	49.5	95.0		88.0	98.0
		50	0.58	30	50.0	94.0		<i>c</i>	<i>c</i>

<sup>a</sup> Calculated by ratio of the area under bound DNA in  $dh/dT$  vs.  $T$  plot divided by area under  $dh/dT$  vs.  $T$  plot for uncomplexed DNA.

<sup>b</sup>  $T_{mf}$  = temperature of melting of free DNA. <sup>c</sup> Melting band present but not peaked.

Complexes of annealed poly(L-Lys<sup>84.5</sup>-L-Val<sup>15.5</sup>)-DNA (LV-DNA) show two distinct melting bands in the bound DNA region at  $r = 0.5$  (Table IV). The hyperchromicity melt of LV-DNA, at several  $r$  ratios, is seen in Figure 5A. At  $r = 0.5$  the melt shows two distinct bands in the bound DNA region, one at  $T_{m1} = 90.6^\circ\text{C}$  and a higher one at  $T_{m2} = 98.2^\circ\text{C}$  (also see Table IV). The second band is not present at  $r = 0.25$ . The presence of these two bands is again confirmed in the  $[\Theta]_{280}$  melt with two corresponding bands at  $T_{m1} = 93^\circ\text{C}$  and  $T_{m2} = 100^\circ\text{C}$ , as seen in the derivative plot of  $d[\Theta]_{280}/dT$  vs.  $T$  (Figure 5B). The relative amounts of the DNA involved in the  $T_{m1}$  and  $T_{m2}$  melts and the appearance of  $T_{m2}$  at  $r = 0.5$  are consistently seen in both hyperchromicity (Figure 5A) and ellipticity (Figure 5B) melting curves. These curves are different than both those of the annealed and directly mixed L-DNA melts (Figure 4).

Complexes of annealed poly(L-Lys)<sup>87.2</sup>-poly(L-Val)<sup>12.8</sup>-DNA (block LV-DNA) show two main melting bands in the hyperchromicity melt-out (Figure 6A, at  $r = 0, 0.25$ , and  $0.5$ ), one for the bound and one for unbound DNA. The unbound DNA melts at  $49.5$ – $50^\circ\text{C}$  while the bound DNA melts at  $94$ – $95^\circ\text{C}$  (Table IV). The thermal denaturation pattern in the ellipticity melt of the block LV-DNA complex also shows two bands at  $r = 0.25$ , shown as  $d[\Theta]_{280}/dT$  vs.  $T$  in Figure 6B (at  $r = 0, 0.25$ , and  $0.5$ ). These melts are not as distinctly seen with the block LV-DNA complex as with the statistical copolymer, LV-DNA. The ellipticity changes at  $T_{m2}$  are negative for both the block and statistical copolymer complexes.

For both statistical and block copolymers, the calculated fraction ( $r$ ) of "bound" base pairs is greater than the nominal input value (see Table IV). This may indicate that the blocks of valine contribute to the stabilization of the DNA double-stranded structure.

For both directly mixed L-DNA and annealed statistical LV-DNA complexes, both the  $T_{m1}$  and  $T_{m2}$  measured by hyperchromicity occur at lower temperatures than found by ellipticity changes. However, the annealed L-DNA shows an ellipticity change prior to hyperchromicity change. Although the reproducibility from sample to sample of  $T_{m1}$  and  $T_{m2}$  is  $\pm 2.0^\circ\text{C}$ , smaller temperature differences between hyperchromic and ellipticity changes can be detected because both spectral quantities are simultaneously measured.

The premelting of statistical LV-DNA is more similar to the annealed L-DNA than the directly mixed complexes perhaps due to some similarities in the aggregation of both these complexes. Increasing  $r$  increases the average premelting slope of the statistical LV-DNA complex but the value of

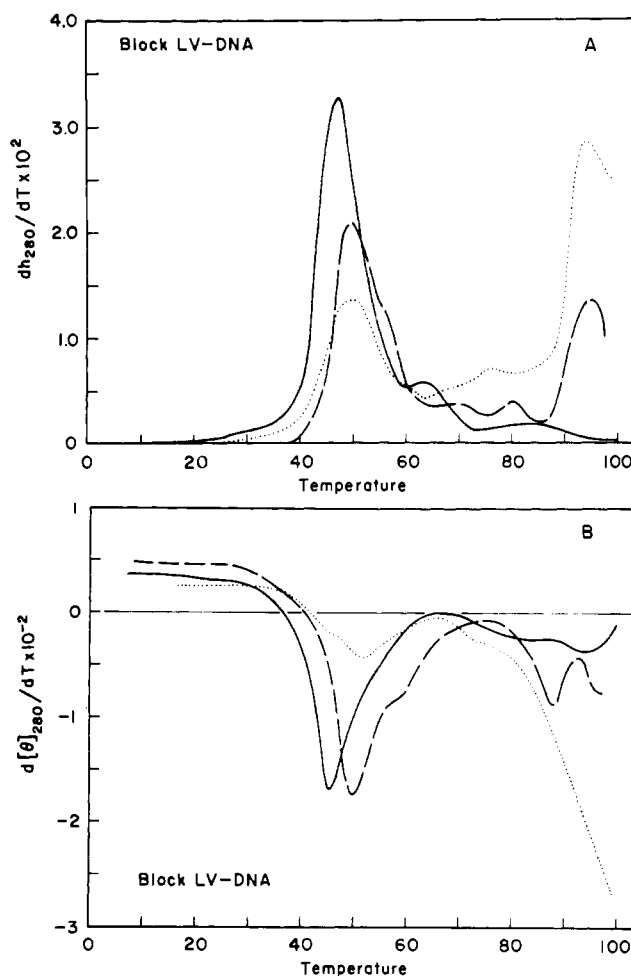


FIGURE 6: Thermal denaturation of annealed complexes of block copolymer poly(L-Lys)<sup>87.2</sup>-poly(L-Val)<sup>12.8</sup>-DNA (block LV-DNA) at  $r = 0.0$  (—);  $r = 0.25$  (---);  $r = 0.50$  (···). (A) Derivative of hyperchromicity with respect to temperature,  $dh_{280}/dT$ , vs. temperature. (B) Derivative of ellipticity with respect to temperature,  $d[\Theta]_{280}/dT$ , vs. temperature.

$[\Theta]_{280}$  is remarkably independent of  $r$  in the statistical LV-DNA complex just prior to melting. On the other hand the premelting slope of block LV-DNA is independent of  $r$ .

#### Discussion

The optical properties of poly(L-Lys)-DNA complexes, as a function of salt, are well known, but little understood. The

CD transition which occurs around 1.0 M NaCl (the critical salt concentration) is characterized by a large, sharp decrease in the value of the ellipticity, having a minimum at 270 nm with  $[\theta]_{270}$  greater than  $1.0 \times 10^5$  for  $r \geq 0.25$ . The complex shows aggregation with visible turbidity, absorption in the ultraviolet at long wavelengths, and a separable precipitate of the complex enriched in poly(L-Lys) with respect to the supernatant (Li et al., 1974c). The large and sharp CD change at  $[\theta]_{280}$  observed as the salt concentration is decreased is paralleled by the precipitability of the DNA in the complex (Sponar et al., 1974) and therefore probably corresponds to the onset of binding between polypeptide and DNA.

The incorporation of L-Val, statistically distributed, into the L-Lys polypeptide shifts the dependence of the CD spectrum change to lower salt concentration and decreases the magnitude of the change (Figure 2). This implies that the LV-DNA complex, although aggregation occurs, alters the long-range order found in L-DNA complexes, which must be changed or disrupted by the L-Val. However, the CD change in LV-DNA may reflect the packing and order of the complex and not the secondary structure of the DNA.

The CD curves for L-DNA and poly(L-Lys<sup>100f</sup>-L-Val<sup>f</sup>)-DNA at low salt ( $2.5 \times 10^{-4}$  M EDTA, pH 7.0) show a less negative  $[\theta]_{280}$  and appear more like native DNA when compared with the high salt spectra. The turbidity of the complexes, as measured by 400-nm absorbance, has also decreased. Complexes of L-DNA at  $r = 0.50$  have CD curves similar to the C-form DNA (Tunis-Schneider and Maestre, 1970), whereas complexes of LV-DNA, at  $r = 0.5$ , show only smaller decreases in  $[\theta]_{280}$ . Block LV-DNA shows even less of a change in  $[\theta]_{280}$  and has a CD similar to that of native DNA at all salt concentrations. These spectral differences may not be due to alterations in the DNA secondary structure, however, but may reflect the packing and long-range order of the DNA. This structural variation can arise directly from differences in the polypeptide secondary structure or due to differing specificities and binding sites for valine than for lysine on the DNA or both. These differences can either be manifested as an A-T or G-C binding preference, a sequence specificity on the DNA, spaced vs. adjacent packing, etc. The specific binding of the polypeptide could then give rise to secondary structural changes in the DNA and in the polypeptide, as well as tertiary changes in the packing and long-range order of the complex, the latter being more probable.

The secondary structure of poly(L-Lys) can be altered as a function of pH and salt; it is known to be a random coil at neutral pH and  $\alpha$  helical at high pH and room temperature (Applequist and Doty, 1962; Ciferri et al., 1968), or in the presence of salts, e.g., 0.5 M CaClO<sub>4</sub> (Dearborn and Wetlaufer, 1970). The binding of poly(L-Lys) to DNA is accompanied by a neutralization of the DNA phosphate backbone by the  $\epsilon$ -amino groups. It is therefore possible for these polypeptides to undergo a conformational change upon binding to DNA. Poly(L-Lys<sup>86.8</sup>-L-Val<sup>13.2</sup>) is a random coil at neutral pH in 0.1 M KF ( $[\theta]_{196} = -41,800$ ) and transforms to a  $\beta$  sheet at pH above 10.3 at room temperature (Mandel and Fasman, 1975). The CD spectra of poly(L-Lys<sup>86.8</sup>-L-Val<sup>13.2</sup>)-DNA,  $r = 0.5$  in  $10^{-2}$  M NaF (not shown), would indicate that the bound polypeptide is in a random coil conformation, although there is no a priori reason to expect that the CD spectra of the complex to be additive in DNA and polypeptide ellipticities. It is therefore doubtful that an overall secondary conformational difference in the bound polypeptide, induced by the presence of valine, accounts for the very different optical properties of complexes of poly(L-Lys<sup>100f</sup>-L-Val<sup>f</sup>)-DNA

compared with poly(L-Lys)-DNA complexes. This conclusion is supported by the smaller change in  $[\theta]_{280}$  of poly(L-Lys)<sup>87.2</sup>-poly(L-Val)<sup>12.8</sup>-DNA in which the L-Lys block would not be forced into a  $\beta$  configuration by the  $\beta$  L-Val block (Kubota and Fasman, 1975).

The optical activity of L-DNA complexes at 1.0 M NaCl is very similar to the CD spectrum of  $\Psi$ -type DNA, a condensed state of DNA as a consequence of exclusion from polyethylene oxide-NaCl solutions (Jordan et al., 1972). This type of CD spectra now appears to be an usual and ubiquitous phenomena in DNA, polynucleotides (Brunner and Maestre, 1974), and complexes of proteins and polypeptides with DNA. It has been suggested that the large optical activity is due to the asymmetric packing of the double-stranded helices, reflecting long-range order rather than an altered secondary structure of DNA. In addition, it has been shown that very large ellipticities, in the absorption region, may arise from cholesteric liquid crystal formation (Holzwarth and Holzwarth, 1973). It has been shown, from the x-ray scattering studies (Maniatis et al., 1974) of  $\Psi$ -type DNA, that the DNA is in the B form. The DNA in L-DNA complexes in 1.0 M NaCl, on the basis of unoriented and poorly resolved x-ray diffraction patterns, has also been found to be in the B form (Haynes et al., 1970). More recently polarized infrared measurements (Liquier et al., 1975) have also shown DNA to be in the B form in similar complexes. Therefore, does the CD and thermal denaturation curve at low salt reflect changes in the DNA helix geometry or the interhelical packing and long-range order in the DNA? The above evidence strongly favors the latter, namely the higher order asymmetry due to packing or condensation. When L-Val is incorporated in a random copolymer with L-Lys, it inhibits formation of  $\Psi$ -type DNA, although the copolymer binds almost as well to DNA on the basis of the complex thermal denaturation stability. This observation, coupled with the fact that complexes of poly(L-Lys<sup>84.5</sup>-L-Val<sup>15.5</sup>)-DNA and poly(L-Lys)<sup>87.2</sup>-poly(L-Val)<sup>12.8</sup>-DNA at  $r = 0.5$  have CD spectra similar to that of native DNA at the same ionic strength, implies that the DNA is in the B form and that changes in the CD spectra reflect differences in the packing of the DNA and not the secondary structure. Consequently, as mentioned above, it is also expected that DNA in annealed L-DNA is in the B form. In addition, comparison of the block and statistical LV-DNA complexes shows that runs of L-Val prevent formation of  $\Psi$ -type DNA. The statistical copolymer probably effectively prevents  $\Psi$  formation in the DNA by virtue of blocks of valine in the copolymer.

The  $[\theta]_{280}$  premelting slope of DNA in various NaCl concentrations, as seen in Figure 1 and Table II, is relatively independent of salt concentration and fairly linear with respect to temperature. It has been proposed that this is caused by a transition from a C-like form of DNA to a B-like form, through a continuum of thermodynamic states with essentially zero enthalpy (Gennis and Cantor, 1972). It was suggested that this transition occurs by an unwinding of the DNA prior to melting. However, the following data argue against such a simple interpretation.

A progressive winding of the DNA molecule with increased salt concentration is not supported by the CD data, which shows that at constant temperature the  $[\theta]_{280}$  goes through a maximum around 0.01 M Na<sup>+</sup> (Figure 1 and Table I). It should be noted that this corresponds approximately to the same salt concentration at which the measured helix-coil enthalpy is a maximum (Gruenwedel, 1974). It has been pointed out by Studdert and Davis (1974), using the Johnson and Ti-

noco theory to calculate the CD spectrum of DNA as a function of geometry, that the CD spectrum is much more sensitive to the distance between the base pairs and the helix axis along the dyad axis ( $D$ ) than to the winding angle. The geometry of the B form of DNA (Langridge et al., 1960; Arnott and Hukins, 1972) allows for a range of dyad distance  $D_y$  between 1.68 and 3.16 Å as compared with 0.13 Å for the C form. The x-ray scattering curve of calf thymus DNA in 6.0 M LiCl (Maniatis et al., 1974) indicates that the DNA is essentially B form, although the CD spectrum has no positive peak at 270 nm and looks similar to that of C-form DNA. At high salt, DNA is effectively dehydrated. On the other hand, lowering the temperature increases the hydration (Lubas and Wilczok, 1970). Therefore it is inconsistent for the C form to be more stable at high salt and low temperature. For these reasons the conformation of DNA should probably be considered to be in one form of a family of B forms.

The two annealed complexes (L-DNA and statistical LV-DNA) exhibit very different CD thermal denaturation patterns for the polypeptide bound DNA segments. This probably reflects differences in both the long-range order of the aggregates as well as the specific binding of the polypeptide to DNA. Upon denaturation of double-stranded DNA, one expects a decrease in  $[\theta]_{280}$  (Brahms and Mommaerts, 1964) (see Figure 1, for example). This is due to breaking of the base pair hydrogen bonds, unstacking of the bases, and a separation of the two strands, all yielding greater conformational freedom of the base pairs and a less asymmetric field and weaker coupling of the base electronic transitions. This process probably occurs in several steps as evidenced by a continued ellipticity decrease at temperatures greater than  $T_{m1}$  in DNA (Figure 1), by the higher thermal stability of G-C sequences relative to A-T sequences (Inman and Schnos, 1970) and by the necessity of unwinding the DNA double helix for strand separation to occur (Davison, 1966).

For LV-DNA each transition subsequent to premelting is a negative ellipticity change whereas the most prominent feature of L-DNA is a very large increase in the ellipticity at  $T_{m2} = 95.0^\circ\text{C}$  (Figure 4B). This latter change occurs at a slightly lower temperature than the hyperchromicity change and is followed closely by a sharp decrease in the 280-nm ellipticity. In addition, the magnitude of the ellipticity increase is cooperative (that is nonlinear) with respect to the ratio of lysyl to phosphate. It is proposed, therefore, that this melt is caused by a breakdown of the long-range order just prior to melting of the bound segments. Upon unfolding or partial disaggregation of the helices, the strands then unwind and separate. The end of the latter process is seen by the  $[\theta]_{280}$  decrease which occurs at temperatures slightly higher than  $T_{m2}$  as monitored by hyperchromicity change. This implies that the long-range order is maintained despite the partial denaturation of segments.

Comparison of the thermal denaturation pattern of chromatin (Henson and Walker, 1970; Wilhelm et al., 1974) with the complexes examined in this paper offers another possible explanation for the decreased  $[\theta]_{280}$  band. The denaturation of chromatin monitored by CD shows a rapidly increasing  $[\theta]_{280}$  centered at  $60^\circ\text{C}$  followed by a decrease at  $75^\circ\text{C}$ . The hyperchromic melting curve (Wilhelm et al., 1974) is not well resolved but shows approximately 20% denaturation at  $60^\circ\text{C}$  and 60% denaturation at  $75^\circ\text{C}$ . Free DNA melts at  $43^\circ\text{C}$  at the solvent conditions used. The peak ellipticity occurs at  $70^\circ\text{C}$  and is approximately  $8400^\circ$ . A similar pattern of melting is seen only on bound segments of DNA in annealed L-DNA complexes. In this complex it appears that the large increase

in  $[\theta]_{280}$  is a result of unfolding and destruction of the long-range order. Therefore an alternative interpretation of the decreased  $[\theta]_{280}$  in chromatin and PS particles (Rill and Van Holde, 1973) is that it reflects the tertiary folding of the protein-DNA complex which does not exclude the secondary structure of DNA from being in B form. The cooperative thermal transition may then reflect unfolding of the tertiary structure rather than a C to B transition.

Comparison of the results herein with studies of interactions between DNA and polypeptide containing, in addition to L-lysine, L-proline, L-alanine, and L-glycine (Sponar et al., 1973, 1974) or L-leucine (Ong and Fasman, 1976; Ong et al., 1976) shows the following pattern. If there are long runs or a large fraction of hydrophobic groups, aggregates form with large positive ellipticities at 280 nm. If there is a large run of lysine with neutral amino acids (glycine or proline),  $\Psi$ -type DNA spectra are found (large negative ellipticity). However, at the ratios of L-lysine to L-valine used in this study, neither effect is dominant and there is little change in the circular dichroism. Thus the nature of the hydrophobic side chain is important.

The CD of the annealed poly(L-Lys)-DNA complexes at salt concentrations below 1.0 M is attributed to a combination of long-range order (tertiary structure) and the secondary structure of DNA. The complexes containing valine are not capable of the same long-range order found in the poly(L-Lys)-DNA complexes. This is reflected in the lack of positive  $[\theta]_{280}$  change and relatively undistorted CD spectra, though the CD distortion at high salt may reflect folding in the aggregate. It is probable that the statistical copolymers of L-Lys and L-Val are not very random in their distribution, but rather have long runs of L-Val which is responsible for the inhibition of the  $\Psi$ -type spectra.

The ionic-strength dependence of the changes of  $[\theta]_{280}$  of the complex demonstrates the importance of the role that nonionic interactions (hydrophobic) play in the association between DNA and polypeptides and proteins. These interactions and the interactions between the bound polypeptide determine the behavior of the complexes, such as causing condensation into higher order asymmetric structures, i.e., the tertiary organization.

In conclusion, the use of thermal denaturation, in which both ellipticity and absorption are measured, yields significant information regarding the structure and stability of the DNA complexes with polypeptides and proteins. They are somewhat complementary probes insofar as hyperchromicity measurements directly yield information about the base stacking and base pairing, whereas CD yields information concerning both the secondary structure and long-range order of the complex, i.e., folding into superhelices, etc.

This study has further demonstrated that the amino acid composition and sequence of polypeptides bound to DNA play an important role in the manner in which the complex is condensed into higher ordered structures, such as is found in chromatin.

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