Helix–Coil Transition and Conformational Studies of Nucleoprotein: Poly(L-arginine)- and Poly(L-arginine^{87}, L-ornithine^{13})–DNA Complexes. II. Circular Dichroism†

Sharon S. Yu, Paul Epstein, and Hsueh Jei Li*

ABSTRACT: Circular dichroism (CD) of DNA with varied GC contents, synthetic copoly(dAT) and poly(dG)·poly(dC) complexed with polyarginine, and calf thymus DNA complexed with poly(L-arginine^{87}, L-ornithine^{13}) in 2.5 × 10^{-4} M EDTA (pH 8.0) has been studied. For natural DNA, the binding of polyarginine or poly(Arg^{87},Orn^{13}) yields red shifts for λ_{max} of the positive band at 275 nm and λ_c of the crossover at 255 nm, and a reduction of amplitude of the positive band. The CD spectra of the complexes can always be decomposed into two components, $\Delta\epsilon_0$ of free base pairs equal to that of free DNA in B conformation and $\Delta\epsilon_b$ of bound base pairs with a conformation between B and C structures. For copoly(dAT), the CD of the complex with polyarginine can also be decomposed into two components, $\Delta\epsilon_0$ of free and $\Delta\epsilon_b$ of bound base pairs. Both $\Delta\epsilon_0$ and $\Delta\epsilon_b$ of polyarginine–copoly(dAT) complexes are quite different from their counterparts in polyarginine–natural DNA complexes. For poly(dG)·poly(dC), the binding of polyarginine yields only small CD changes which do not resemble those of natural DNA or copoly(dAT). There are good correlations between thermal denaturation and CD results of these complexes which suggest the requirements of GC content and secondary structure of DNA for a specific binding by polyarginine.

Circular dichroism (CD) has been used in conformational studies of DNA complexed with polylysine (Haynes *et al.*, 1970; Carroll, 1972; Chang *et al.*, 1973), polyarginine (Carroll, 1972), protamine (Yu and Li, 1973), histone I (Olins, 1969; Fasman *et al.*, 1970, 1971), histone IV (Wagner, 1970; Shih and Fasman, 1971; Li *et al.*, 1971), histone IIb2 (Adler *et al.*, 1974; Leffak *et al.*, 1974), and whole histones in chromatin (Shih and Fasman, 1970; Permogorov *et al.*, 1970; Simpson and Sober, 1970; Johnson *et al.*, 1972; Li *et al.*, 1974b). Previously only CD of the whole protein–DNA complex could be dealt with. Recently, by using thermal denaturation as an independent method to determine the fraction of base pairs bound by proteins, we have been able to obtain CD or conformation of protein-bound base pairs in a nucleoprotein, such as polylysine–DNA (Chang *et al.*, 1973), protamine–DNA complex (Yu and Li, 1973), nucleohistone IIb2 (Leffak *et al.*, 1974), and chromatin (Li *et al.*, 1974b).

As an extension of our previous work we report here the CD results of polyarginine complexed with DNA of varied gc contents and poly(Arg^{87},Orn^{13})–DNA complexes. As shown in the previous report (Epstein *et al.*, 1974), there are two melting bands at around 100° ($T_{m,I}'$ and $T_{m,II}'$), corresponding to polyarginine-bound base pairs. Melting temperatures and amplitudes of these two melting bands depend upon the GC content and the secondary structure of DNA, and the presence of ornithine in polyarginine. Though the melting properties appear to vary greatly in these complexes, the differences in CD are not so obvious. In each complex, except for poly(dG)·poly(dC), by taking the average of CD of polyarginine- or poly(Arg^{87},Orn^{13})-bound bases, irrespective of the presence of $T_{m,I}'$ and $T_{m,II}'$, the CD of each complex can also be decomposed into $\Delta\epsilon_0$ of free and $\Delta\epsilon_b$ of bound base pairs. For poly(dG)·poly(dC), the addition of polyarginine does not induce any significant CD change.

Materials and Methods

Materials and methods, except those in CD, are identical with those in Epstein *et al.* (1974). The CD spectra of DNA, polyarginine–DNA, and poly(Arg^{87},Orn^{13})–DNA complexes were taken on a Durrum-Jasco spectropolarimeter, Model J-20, at room temperature in 2.5 × 10^{-4} M EDTA (pH 8.0), the buffer used for thermal denaturation. The CD results are reported as $\Delta\epsilon = \epsilon_l - \epsilon_r$, where ϵ_l and ϵ_r are respectively molar extinction coefficients for the left- and the right-handed circularly polarized light. The units of $\Delta\epsilon$ are M^{-1} cm^{-1} in terms of nucleotide. The concentrations of the complexes were determined by using the same molar extinction coefficients at 260 nm as those in pure DNA (Epstein *et al.*, 1974).

Results

CD Spectra of Calf Thymus DNA Complexed with Polyarginine and poly(Arg^{87},Orn^{13}). Figure 1 shows CD spectra of calf thymus DNA complexed with various amounts of polyarginine. As more polyarginine is bound to DNA, there are red shifts for both the positive band near 275 nm and the crossover near 255 nm. The amplitude of the positive band is also reduced. These CD changes on DNA due to polyarginine binding are essentially the same as those found in direct mixed polylysine–DNA (Chang *et al.*, 1973) and protamine–DNA complexes (Yu and Li, 1973).

When the difference CD spectra of pure DNA and the complexes were taken it was found that the shapes are identical and the amplitude of each difference CD spectrum is proportional to the *B* value (input ratio of amino acid residue per nucleotide) in that complex. It indicates that the CD of the complex, $\Delta\epsilon_m$, can be decomposed into two components, $\Delta\epsilon_0$ of free and $\Delta\epsilon_b$ of bound base pairs, as done before in polylysine–DNA (Chang *et al.*, 1973) and protamine–DNA complexes (Yu and Li, 1973).

As shown earlier (Epstein *et al.*, 1974), there are two melting bands at T_m' in polyarginine–DNA complexes, indicating

† From the Department of Chemistry, Brooklyn College of the City University of New York, Brooklyn, New York 11210. *Received March 14, 1974.* Supported by National Science Foundation Grant GB35459, U. S. Public Health Service Grant GM20596, and Research Foundation of the City University of New York.

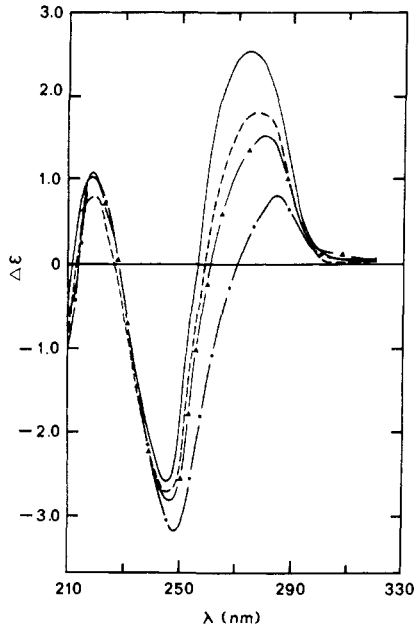

FIGURE 1: CD spectra of polyarginine-calf thymus DNA complexes. $B = 0$ (——), 0.20 (- - -), 0.35 (- ▲ -), and 0.60 (— · —).

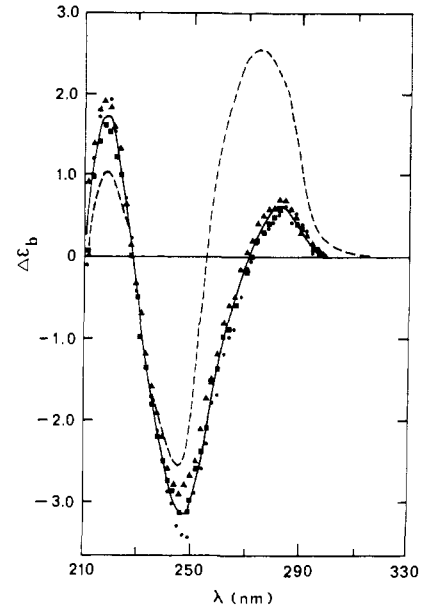

FIGURE 3: Calculated CD spectrum ($\Delta\epsilon_b$) of calf thymus DNA base pairs bound by poly(Arg^{87},Orn^{13}). B = 0.40 (△), 0.60 (●), and 0.80 (■). Also included is $\Delta\epsilon_0$ of calf thymus DNA (- - - -).

two types of bound base pairs. For the analysis of CD results of the complexes, the average CD, $\Delta\epsilon_b$, of these two types of bound base pairs is used. In other words, it is assumed that, for each complex, the free base pairs have $\Delta\epsilon_0$ equal to that of pure DNA and the bound base pairs have an average CD, $\Delta\epsilon_b$, which is to be determined. As defined above, $\Delta\epsilon_m$ is the measured CD of the complex. If F is the fraction of base pairs bound in each complex, we obtain

$$\Delta\epsilon_m = (1 - F)\Delta\epsilon_0 + F\Delta\epsilon_b \qquad (1)$$

where F is determined by thermal denaturation from eq 1 in Epstein *et al.* (1974). This method is identical with the one used before (Chang *et al.*, 1973). From eq 1, $\Delta\epsilon_b$ of bound base pairs in each complex can be determined. The calculated $\Delta\epsilon_b$ for three polyarginine-calf thymus DNA complexes are shown in Figure 2. Within experimental error these three complexes have the same $\Delta\epsilon_b$ with λ_{max} of the positive band at 286 nm, λ_c of the crossover at 278 nm, and $\Delta\epsilon_{286} = 0.53$ in contrast to $\Delta\epsilon_0$ of pure DNA with λ_{max} at 275 nm, λ_c at 256.5 nm, and $\Delta\epsilon_{275} = 2.53$. This $\Delta\epsilon_b$ is close to that of polylysine-bound base pairs, with λ_{max} 283 nm, λ_c 273 nm, and $\Delta\epsilon_{283} = 0.80$ (Chang *et al.*, 1973). It implies the existence of a conformation between B and C structures for polyarginine-bound base pairs (Tunis-Schneider and Maestre, 1970; Chang *et al.*, 1973).

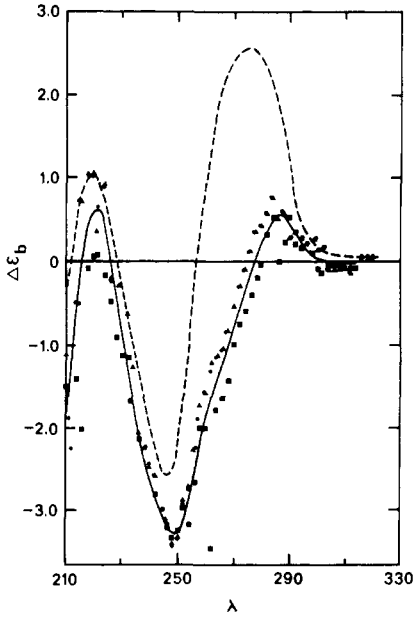

FIGURE 2: Calculated CD spectrum ($\Delta\epsilon_b$) of calf thymus DNA base pairs bound by polyarginine. B = 0.20 (■), 0.35 (▲), and 0.60 (●). Also included is $\Delta\epsilon_0$ of calf thymus DNA (- - -).

The CD spectra of calf thymus DNA complexed with poly(Arg^{87},Orn^{13}) are similar to those of polyarginine-calf thymus DNA complexes (Figure 1) except at 220-nm region, though the thermal denaturation results at T_m' are very different in these two complexes (Figures 2 and 8 in Epstein *et al.*, 1974). Using eq 1, $\Delta\epsilon_b$ from these complexes are calculated and shown in Figure 3. Again they agree with one another and have one $\Delta\epsilon_b$ with λ_{max} at 282 nm, λ_c at 271 nm, and $\Delta\epsilon_{282} = 0.63$. Compared with $\Delta\epsilon_b$ of polyarginine-DNA, it is seen that $\Delta\epsilon_b$ of poly(Arg^{87},Orn^{13})-DNA is closer to that of polylysine-DNA (Chang *et al.*, 1973) except that, at 220 nm, $\Delta\epsilon_b$ is much greater for poly(Arg^{87},Orn^{13})-DNA complexes. This is in agreement with thermal denaturation data that the presence of ornithine makes polyarginine-DNA appear closer to polylysine-DNA (Epstein *et al.*, 1974).

CD Spectra of Bacterial DNA Complexed with Polyarginine. In the previous report (Epstein *et al.*, 1974), it was shown that thermal melting properties of polyarginine-DNA complexes depend upon the GC content of DNA used. Bacterial DNA of varied GC contents were used here for testing such a possible CD dependence, $\Delta\epsilon_b$ in particular, on the GC content.

Similar to those observed for polyarginine-calf thymus DNA complexes, same types of CD changes are observed when polyarginine is complexed to bacterial DNA. Figure 4 shows $\Delta\epsilon_0$ of pure *Micrococcus luteus* DNA and the calculated $\Delta\epsilon_b$ of polyarginine-bound *M. luteus* DNA. The CD spectrum of pure *M. luteus* DNA is essentially the same as that reported by Gratzer *et al.* (1970). For the CD of complexed DNA, $\Delta\epsilon_b$, the red shift of λ_{max} and λ_c, and a reduction of the amplitude of the positive CD band near 270 nm and an enhancement of the amplitude of the negative band near 245 nm are exactly the

TABLE I: Circular Dichroism Parameters of Polyarginine–Bacterial DNA Complexes.[a]

DNA	$\Delta\epsilon_0$					$\Delta\epsilon_b$				
	λ_{max}	λ_{min}	λ_c	$\Delta\epsilon_{\lambda_{max}}$	$\Delta\epsilon_{\lambda_{min}}$	λ_{max}	λ_{min}	λ_c	$\Delta\epsilon_{\lambda_{max}}$	$\Delta\epsilon_{\lambda_{min}}$
M. luteus	270	244	256	2.7	−2.1	279	248	265	0.7	−2.7
E. coli	276	247	258	2.5	−2.6	285	248	276	0.4	−3.5
Cl. perfringens	273	246	256	3.2	−2.4	279	247	263	0.8	−3.2

[a] λ_{max}, λ_{min}, and λ_c are respectively the wavelengths (nm) of the maximum, the minimum, and the crossover of the CD spectra.

same as those for calf thymus DNA. Similar CD results are also found for polyarginine-*Escherichia coli* DNA and polyarginine-*Clostridium perfringens* DNA complexes. The pertinent CD parameters of these complexes are summarized in Table I.

CD Spectra of Synthetic DNA Complexed with Polyarginine. Synthetic double-stranded copoly(dAT) has a CD spectrum (Figure 5) different from those of natural DNA from calf thymus or bacteria. For instance, the CD of copoly dAT has λ_{max} at 264 nm and a shoulder at about 280 nm. Natural DNA from calf thymus or bacteria has λ_{max} at 270–275 nm. In addition, the amplitudes of the negative band at about 245 nm and the second positive band at about 220 nm are much bigger for copoly(dAT) than for natural DNA. These differences are not solely due to their AT contents. For instance, Gratzer *et al.* (1970) calculated CD spectra of DNA with 100% AT based upon the CD spectra of natural DNA with varied AT or GC contents. Our CD spectrum of copoly(dAT) is similar to that calculated by Gratzer *et al.* (1970) at λ <250 nm but is quite different at λ >250 nm. For instance, the calculated CD has λ_{max} 280 nm and λ_c 261 nm, compared with the measured CD with λ_{max} 264 nm and λ_c 255 nm. We attribute this CD difference to the difference in secondary structure of synthetic and the hypothetical natural DNA with 100% AT which will be discussed later.

Addition of polyarginine to copoly(dAT) shows a slight red shift for the crossover (λ_c) at 255 nm and a reduction of the amplitude of the positive CD band near 265 nm. These are similar to those of natural DNA. In contrast to natural DNA, the red shift for the positive band near 265 nm is not apparent for copoly(dAT) (Figure 5). When the difference CD spectra were taken it was found that they were identical for two complexes with different B values and the amplitude was proportional to the fraction of base pairs bound in the complex. Therefore, eq 1 was used for the calculation of $\Delta\epsilon_b$ and the results are shown in Figure 6. As expected, these two complexes with B = 0.90 and 1.6 have the same $\Delta\epsilon_b$. The shape of $\Delta\epsilon_b$ for polyarginine-bound copoly(dAT) base pairs is very different from those when natural DNA were used. $\Delta\epsilon_b$ in copoly(dAT) has a peak with zero amplitude at 263 nm and a trough at 270 nm.

Double-stranded poly(dG)·poly(dC) has a CD spectrum (Figure 7) very different from those of natural DNA and copoly(dAT). There is a big positive peak at 255 nm, an intermediate positive peak at 270 nm, and another peak with negative amplitude at 224 nm. Again, this CD spectrum is similar to the calculated CD spectrum of a hypothetical DNA with 100% GC (Gratzer *et al.*, 1970). They are completely different, however, at λ >250 nm. As polyarginine is added to poly(dG)·poly(dC), there are no CD changes typical of those of other DNA, namely red shifts for λ_{max} (except copoly(dAT)) and λ_c and a reduc-

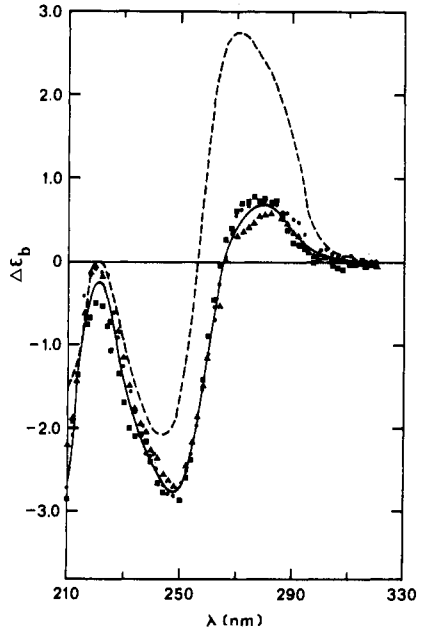

FIGURE 4: Calculated spectrum ($\Delta\epsilon_b$) of *M. luteus* DNA base pairs bound by polyarginine. B = 0.35 (■), 0.60 (●), and 0.70 (▲). Also included is $\Delta\epsilon_0$ of *M. luteus* DNA (- - - -).

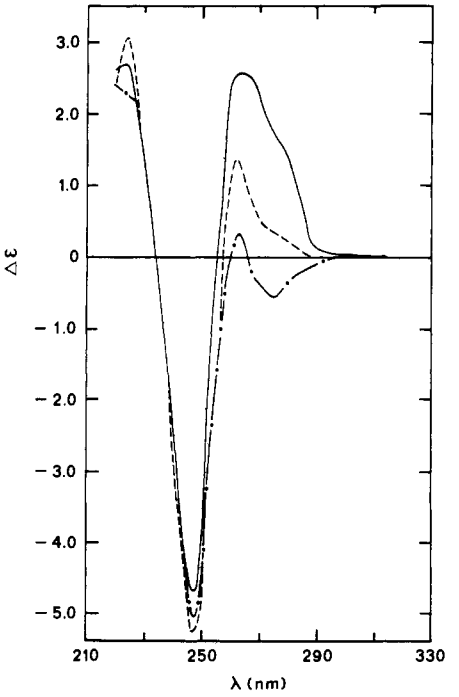

FIGURE 5: CD spectra of polyarginine-copoly(dAT) complexes. B = 0 (——), 0.90 (- - -), and 1.6 (— · —).

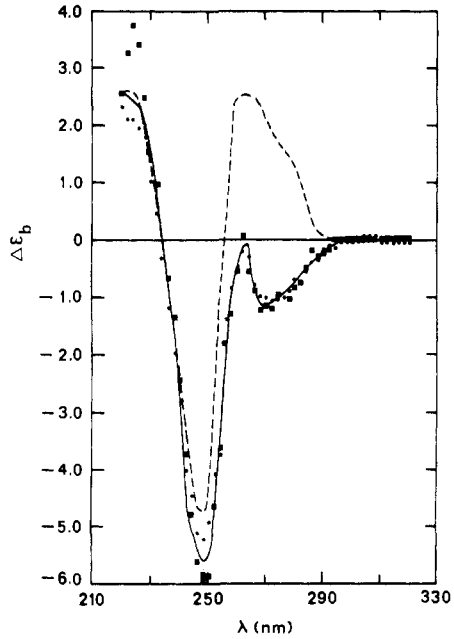

FIGURE 6: Calculated CD spectrum ($\Delta\epsilon_b$) of copoly(dAT) base pairs bound by polyarginine. B = 0.90 (■) and 1.60 (●).

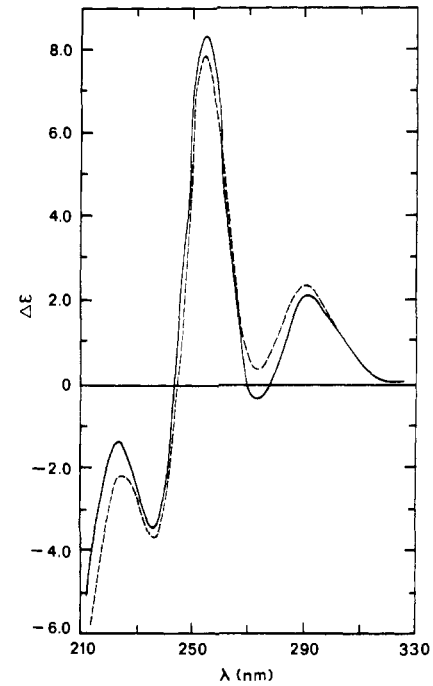

FIGURE 7: CD spectra of polyarginine-poly(dG)·poly(dC) complexes. B = 0 (——) and 0.60 (- - -).

tion of the positive band near λ_{max}. On the contrary, the presence of polyarginine (0.6 arginine/nucleotide) slightly reduces the amplitude of 255 nm and enhances the amplitude at 270 nm (Figure 7). The presence of an intermediate level of polyarginine (0.4 arginine/nucleotide) yields a CD spectrum between the two shown in Figure 7. In other words, the small CD changes in poly(dG)·poly(dC) is also a function of the amount of polyarginine added.

Discussion

It has been demonstrated that, in a nucleoprotein, it is possible to divide the molecule into free and protein-bound base pairs with various thermal stabilities (Li, 1973). One of the most important applications of this view is in the experiments of pea bud nucleohistone (Li and Bonner, 1971; Li, 1972) and calf thymus nucleohistone (Li *et al.*, 1973), with the proposal that a histone molecule with uneven distribution of basic amino acid residues, when bound on DNA in nucleohistone, induces two phases of melting on DNA. Another important application is the calculation of CD of protein-bound base pairs in a nucleoprotein, such as polylysine-DNA (Chang *et al.*, 1973), protamine-DNA (Yu and Li, 1973), and chromatin (Li *et al.*, 1974b). Again the model of helix-coil transition (Li, 1973) is used here as an analytical tool to calculate the CD of polyarginine-bound base pairs.

As seen from the present report, interaction between a protein and DNA is indeed a very complicated problem. In order to come to a reliable conclusion or proposal on this interaction, sufficient experimental facts by varying parameters, such as proteins, DNA, ionic environments, etc., are needed. The use of polyarginine, poly(Arg^{87},Orn^{13}), and DNA of varied GC contents and secondary structures in this report is an example of this approach.

Previously it was thought that ionic interaction between a protein and a DNA was nearly the sole factor for thermal stability of the complex. Recently this thought was modified that hydration in a protein-bound region (Li *et al.*, 1974a) and the binding of a protein in the major or minor groove of DNA (Epstein *et al.*, 1974) could also have a significant effect on thermal stability of the bound region. It is also revealed here that double-stranded DNA, such as poly(dG)·poly(dC), when added with polyarginine, does not guarantee conformational changes similar to that of a transition from B to C structure, as has always been found for other DNA. Apparently there are structural requirements on DNA for a tight winding and binding by a basic polypeptide such as polyarginine. Such a structural requirement for a specific binding of polyarginine on DNA is also supported by thermal denaturation results when double-stranded copoly(dAT) and poly(dG)·poly(dC) are used (Epstein *et al.*, 1974).

As shown in Figures 5 and 7 the CD of copoly(dAT) and poly(dG)·poly(dC) are different from those of natural DNA from calf thymus or bacteria. Though difference in base sequence among those natural and synthetic DNA can cause some variations in CD spectra (Johnson and Tinoco, 1969; Gratzer *et al.*, 1970) the comparison of these two CD spectra of synthetic DNA and those counterparts in hypothetical natural DNA with 100% AT and 100% GC (Gratzer *et al.*, 1970) indicated that the secondary structures of these two synthetic DNA are quite different from those of natural DNA, presumably in B conformation. The extrapolation method used by Gratzer *et al.* (1970) is valid only when all the DNA used with varied GC contents have the same secondary structure, namely B form. Otherwise, a variation in the secondary structure alone can cause a big CD change. This comment is in agreement with the report using X-ray diffraction that sodium salt of copoly(dAT) has D form (Davies and Baldwin, 1963), which is quite different from the B structure of natural DNA. In conjunction with this discussion, the difference between $\Delta\epsilon_b$ of polyarginine-copoly(dAT) complexes (Figure 6) and those of polyarginine-natural DNA can be explained as a result of a difference in their secondary structures.

Although the melting properties of polyarginine-DNA complexes at T_m' are different among natural DNA of varied GC contents (Epstein *et al.*, 1974), their CD properties are more or less the same. At least it is fair to say that within experimental

error there is no significant variation in $\Delta\epsilon_b$ which corresponds to the difference in T_m' of these complexes. This is an example showing that thermal denaturation is a more sensitive method than CD in the study of a protein–DNA complex. Of course, in other cases, such as reconstituted and direct mixed polylysine–DNA complexes, the variation in CD (Carroll, 1972; Chang *et al.*, 1973) is much more obvious than in thermal denaturation (Tsuboi *et al.*, 1966; Olins *et al.*, 1967; Li *et al.*, 1973, 1974a). These examples show that both thermal denaturation and CD, when used together for studying a nucleoprotein, provide information complementary to each other.

References

Adler, A. J., Ross, D. G., Chen, K., Stafford, P. A., Woiszwillo, M. J., and Fasman, G. D. (1974), *Biochemistry 13,* 616.

Carroll, D. (1972), *Biochemistry 11,* 421.

Chang, C., Weiskopf, M., and Li, H. J. (1973), *Biochemistry 12,* 3028.

Davies, D. R., and Baldwin, R. L. (1963), *J. Mol. Biol. 6,* 251.

Epstein, P., Yu, S. S., and Li, H. J. (1974), *Biochemistry 13,* 3706.

Fasman, G. D., Schaffhausen, B., Goldsmith, L., and Adler, A. (1970), *Biochemistry 9,* 2814.

Fasman, G. D., Valenzula, M. S., and Adler, A. J. (1971), *Biochemistry 10,* 3795.

Gratzer, W. B., Hill, I. R., and Owen, R. J. (1970), *Eur. J. Biochem. 15,* 209.

Haynes, M., Garrett, R. A., and Gratzer, W. B. (1970), *Biochemistry 9,* 4410.

Johnson, R. S., Chan, A., and Hanlon, S. (1972), *Biochemistry 11,* 4347.

Johnson, W. C., Jr., and Tinoco, I., Jr. (1969), *Biopolymers 8,* 715.

Leffak, I. M., Hwan, J. C., Li, H. J., and Shih, T. Y. (1974), *Biochemistry 13,* 1116.

Li, H. J. (1972), *Biopolymers 11,* 835.

Li, H. J. (1973), *Biopolymers 12,* 287.

Li, H. J., and Bonner, J. (1971), *Biochemistry 10,* 1461.

Li, H. J., Brand, B., Rotter, A., Chang, C., and Weiskopf, M. (1974a), *Biopolymers* (in press).

Li, H. J., Chang, C., Evagelinou, Z., and Weiskopf, M. (1974b), *J. Mol. Biol.* (submitted for publication).

Li, H. J., Chang, C., and Weiskopf, M. (1973), *Biochemistry 12,* 1763.

Li, H. J., Isenberg, I., and Johnson, W. C., Jr. (1971), *Biochemistry 10,* 2587.

Olins, D. E. (1969), *J. Mol. Biol. 43,* 439.

Olins, D. E., Olins, A. L., and von Hippel, P. H. (1967), *J. Mol. Biol. 24,* 157.

Permogorov, U. I., Debabov, U. G., Sladkova, I. A., and Rebentish, B. A. (1970), *Biochim. Biophys. Acta 199,* 556.

Shih, T. Y., and Fasman, G. D. (1970), *J. Mol. Biol. 52,* 125.

Shih, T. Y., and Fasman, G. D. (1971), *Biochemistry 10,* 1675.

Simpson, R. B., and Sober, H. (1970), *Biochemistry 9,* 3103.

Tsuboi, M., Matsuo, K., and Ts'o, P. O. P. (1966), *J. Mol. Biol. 15,* 256.

Tunis-Schneider, M. J. B., and Maestre, M. F. (1970), *J. Mol. Biol 52,* 521.

Wagner, T. E. (1970), *Nature (London) 227,* 65.

Yu, S. S., and Li, H. J. (1973), *Biopolymers 12,* 2777.