Studies on Interaction between Poly(L-lysine^{40},L-alanine^{60}) and Deoxyribonucleic Acids†

Margaret F. Pinkston and Hsueh Jei Li*

ABSTRACT: Both primary and secondary structures of a protein are important in its interaction with DNA. In order to understand the roles played in this interaction by hydrophobic aliphatic amino acid residues, and by α-helical structure in a protein, a random copolymer of 40% L-lysine and 60% L-alanine, poly(Lys^{40},Ala^{60}), was used as a model protein. Complexes between this copolymer and DNA were made by direct mixing in 2.5×10^{-4} M EDTA (pH 8.0) and studied by the methods of circular dichroism (CD) and thermal denaturation. In the free state, poly(Lys^{40},Ala^{60}) possesses α-helical structure. When it is complexed with DNA, the CD spectrum of this copolypeptide near 220 nm is greatly altered, suggesting a distortion of its α-helical structure. This might possibly be caused by strong interaction between the DNA and the amide groups of the protein. When compared with the effect of polylysine binding to DNA, the presence of alanine in this copolypeptide reduces the capacity of polylysine to induce a conformational transition from B to C form, perhaps because of the presence of α-helical structure. If complexes made in EDTA buffer without salt are transferred from low to intermediate ionic environment (0.1 to 0.2 M NaCl, for example), they undergo a further transition toward A form DNA. Such conformational transition is considered to be cooperative, since it was observed to depend upon r^2 rather than r, where r is the input ratio of copolypeptide to DNA. Thermal denaturation studies show that the binding of poly(Lys^{40},Ala^{60}) to DNA in EDTA buffer induces a biphasic melting with a T_m (the melting temperature of free base pairs) at about 50° and a T_m' (melting temperature of bound base pairs) at about 93°; this is lower than the corresponding T_m' (99–101°) of polylysine-bound base pairs. In addition the T_m' of copolypeptide-bound base pairs is shifted to higher temperature at higher ionic strength. Both facts seem to imply that the presence of a rigid α helix in the copolypeptide prevents its lysine residues from fully interacting with phosphates on DNA, despite 3.0 amino acid residues, or 1.2 lysine per nucleotide in the bound regions as determined by thermal denaturation.

Two important techniques in the study of protein–DNA interaction as it relates to gene regulation and the packing of DNA in chromosomes are thermal denaturation and circular dichroism (CD). Thermal stability of protein-bound base pairs is contributed primarily by electrostatic interaction between basic residues and phosphates on the DNA; the conformation of both protein and DNA is manifested by the CD spectra. Through these techniques significant progress has been made in understanding chromatin, histone–DNA, protamine–DNA, and other basic polypeptide–DNA complexes. However, many questions remain unanswered due to the complicated systems involved.

For purposes of simplification, a series of investigations were undertaken in this laboratory, using DNA from various sources complexed with polylysine or polyarginine, or with a synthetic copolypeptide containing one of these basic residues linked randomly to some other single nonbasic type residue. By limiting the copolypeptide in this way, specific control of the variable in a protein, namely the nonbasic residue, is permitted, so that the role of each amino acid residue in protein–DNA interaction can be examined unambiguously. The importance of this study stems from the fact that variation among histones occurs not only in the distribution of lysine and arginine, but also in nonbasic residues. The potential role of chromosomal nonhistone proteins as genetic regulators in eukaryotes (Teng *et al.*, 1971; Stein *et al.*, 1974) gives further stimulus to the need for studying the contribution of nonbasic amino acid residues in protein–DNA interaction.

Very recently Sponar *et al* (1974) studied interaction between DNA and sequential copolymers of three amino acid residues, L-alanine, L-lysine, and L-proline. Using NaCl gradient dialysis for complex formation, they reported dependence of the CD properties of these complexes upon the sequence of the copolymer used. As will be discussed later, interpretation of the CD properties of the complex, in terms of structural alteration of either protein or DNA by binding, is more difficult when the method of reconstitution is used rather than that of direct mixing. Complex formation in the present study was by the latter method. In addition, the copolypeptides we have studied so far carry random sequence.

Results of a study on the interaction of DNA and a model protein containing aromatic amino acid residues, poly(Lys^{50},Tyr^{50}), were recently reported (Santella and Li, 1974). For the present study we selected another model protein, poly(Lys^{40},Ala^{60}), containing hydrophobic alanine residues which cause the copolymer to form an α helix. This system thus provides an ideal means of investigating the roles both of hydrophobic amino acid residues and of the α-helical structure of a protein when the latter interacts with DNA. The thermal stabilization of DNA caused by the binding of basic residues is reduced by the presence of alanine, or, more likely, by the presence of α-helical structures in a protein; the conformational effect on DNA due to polylysine binding is likewise reduced when alanine is pres-

† From the Department of Chemistry, Brooklyn College of the City University of New York, Brooklyn, New York 11210. *Received July 18, 1974.* Supported by National Science Foundation Grant BMS72-02271 A01, U. S. Public Health Service Grant GM20596, and Research Foundation of the City University of New York.

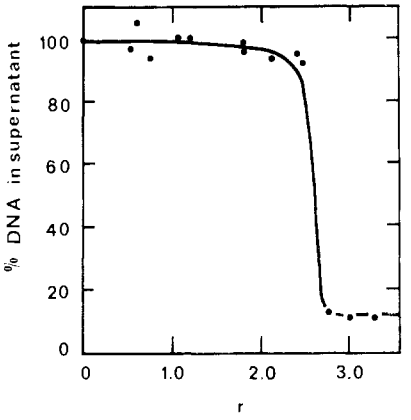

FIGURE 1: Titration precipitation curve of DNA by poly(Lys^{40},Ala^{60}). r = input ratio of amino acid residue to nucleotide.

ent. On the other hand, the α-helical structure of the protein itself is greatly changed by binding. When the complex is transferred from low to intermediate ionic environment another striking effect becomes apparent: a further transition in the secondary structure of DNA to A form is induced, yet the secondary structure of the bound protein is not appreciably altered by the addition of salt. The structural implications of these changes in DNA and protein, and their relation to nucleohistone, are discussed.

Materials and Methods

Random poly(Lys^{40},Ala^{60}), a copolymer of 40% L-lysine and 60% L-alanine, was synthesized using the *N*-carboxyanhydrides of ϵ-carbobenzyloxy-L-lysine and L-alanine (Miles Laboratories) following the methods of Fasman *et al.* (1965) and Morita *et al.* (1967). The amino acid composition of the copolypeptide was checked on a Beckman amino acid analyzer. Concentrations of stock solutions of this copolypeptide were determined by the ninhydrin method (Spies, 1957), using a mixture of 40% L-lysine and 60% L-alanine as the standard.

Calf thymus DNA was purchased from Sigma Chemical Co. and was purified by phenol extraction.

Both DNA and copolypeptide were prepared in 2.5×10^{-4} M EDTA, pH 8.0 (EDTA buffer), before complex formation. Complexes were then made by the slow addition of copolypeptide (10^{-3} M) to DNA (10^{-4} M), and studied by CD and thermal denaturation. Measurement of the titration, or precipitation, curve was the same as described earlier for polylysine–DNA complexes (Li *et al.*, 1973).

To study the effect of ionic strength, a complex was dialyzed overnight against EDTA buffer containing various NaCl concentrations.

DNA concentration was determined spectrophotometrically using a molar extinction coefficient of 6500 M^{-1} cm^{-1} at 260 nm. DNA concentration in the complex was determined using that of free DNA corrected with a dilution factor determined by the quantity of copolypeptide added. When the complex was dialyzed to EDTA buffer containing NaCl, its concentration was determined by measuring A_{260} and correcting further for absorbance due to light scattering, following the method of Leach and Scheraga (1960).

Thermal denaturation measurements were made on a Gilford spectrophotometer Model 2400-S with a constant heating rate of $\frac{2}{3}$°C/min at 260 nm. The hyperchromicity, h, or per cent increase in absorbance, was measured at 260 nm and the derivative of the melting curve, dh/dT, was used to facilitate analysis. CD spectra, taken on a Jasco spectropolarimeter Model J-20, are reported as $\Delta\epsilon = \epsilon_L - \epsilon_R$ in M^{-1} cm^{-1}, where ϵ_L are ϵ_R are molar extinction coefficients for the left- and the right-handed circularly polarized light. For DNA and the complex, M represents moles of nucleotide/liter; for copolypeptide, moles of amino acid residues/liter.

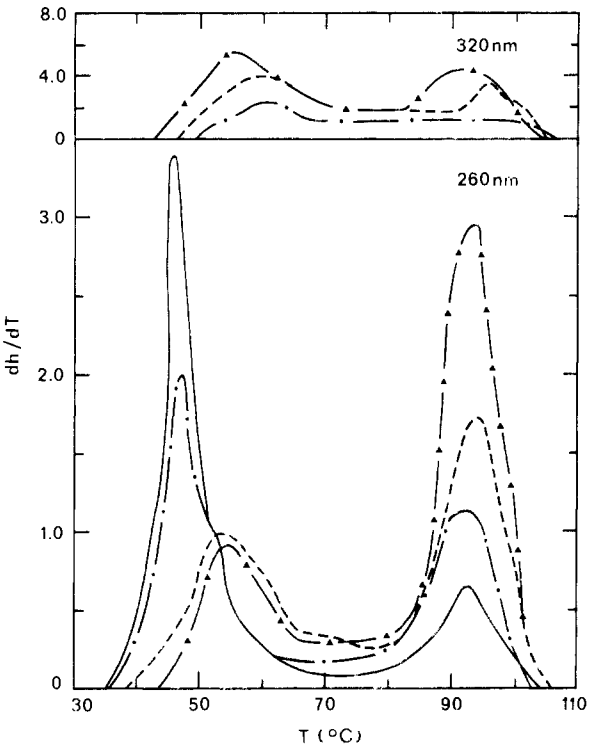

FIGURE 2: Derivative melting profiles of poly(Lys^{40},Ala^{60})–DNA complexes at 260 nm (bottom) and 320 nm (top). r = 0.6 (– –), 1.2 (· –), 1.8 (– – –), and 2.8 (· ▲ –).

Results

Thermal Denaturation and CD of Poly(Lys^{40}, Ala^{60})–DNA Complexes. Figure 1 shows the titration, or precipitation, curve of DNA due to binding of poly(Lys^{40},Ala^{60}). The complex is completely soluble until the input ratio of amino acid residue to nucleotide (r) reaches 2.5 at which point precipitation occurs sharply. The midpoint of precipitation occurs at r = 2.6 which is equivalent to 1.0 lysine/nucleotide, the same as reported for polylysine–DNA complexes (Clark and Felsenfeld, 1971; Li *et al.*, 1973).

Figure 2 shows derivative melting curves of some complexes at 260 and 320 nm. As expected, at 260 nm, as more copolypeptide is bound to DNA, the melting area of free base pairs at about 50° (T_m) is reduced, accompanied by an increase in the melting area of bound base pairs at about 93° (T_m'). Compared with those of polylysine–DNA complexes prepared by the same method in the same buffer (Li *et al.*, 1973), there are three major differences. In polylysine–DNA complexes, the melting occurs exclusively in T_m and T_m' regions, and the hyperchromicity change between them is negligibly small, whereas in poly(Lys^{40},Ala^{60})–DNA complexes the residual melting between these two melting bands is significant, as shown in Figure 2. Secondly, for copolypeptide–DNA complexes of higher r values, there is a rather large upward shift of about 7° in the T_m melting band compared with a shift of approximately 3° in

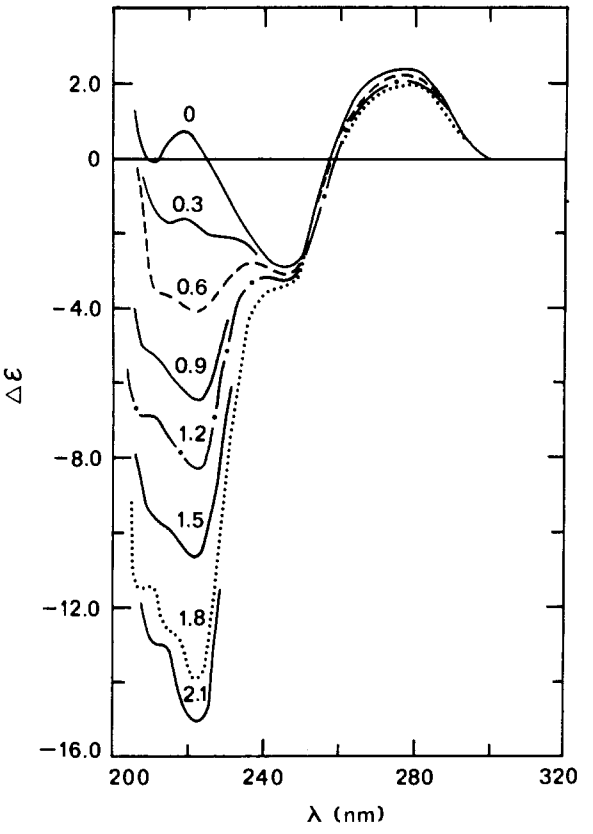

FIGURE 3: CD spectra of poly(Lys^{40},Ala^{60})-DNA complexes. r value of each complex is indicated.

polylysine-DNA complexes. A third significant difference is the fact that, while in polylysine-DNA complexes h_{max} is reduced when r of the complex is increased, in copolypeptide-DNA complexes h_{max} is increased by as much as 6% above that of pure DNA.

These properties, found in poly(Lys^{40},Ala^{60})-DNA but not in polylysine-DNA complexes, can be due to the presence of alanine in the complex which favors some hydrophobic contact among bound regions, intermolecularly or intramolecularly, during melting. If this is the case, some excess hyperchromicity at 260 nm could be contributed by an increase of light scattering. The melting curves monitored at 320 nm, shown in Figure 2, support this explanation, as the hyperchromic change at 320 nm becomes more significant for complexes with higher coverages. Similar phenomena were reported before on reconstituted protamine-DNA complexes (Olins *et al.*, 1968).

Figure 3 shows CD spectra for copolymer-DNA complexes of varied r values. As more copolypeptide is bound to DNA, two significant changes in CD spectra can be seen. The CD band near 220 nm becomes more negative, and the CD band of DNA near 275 nm is slightly reduced and red shifted. The latter effect is similar to that found with polylysine binding but is of much lesser magnitude.

Quantitative Analysis of Thermal Denaturation and CD Results. Previously thermal denaturation results were used to determine the fraction of protein-bound base pairs in a nucleoprotein; this fraction, in turn, was used to calculate the CD of protein-bound base pairs, for polylysine-DNA (Chang *et al.*, 1973), protamine-DNA (Yu and Li, 1973), and polyarginine-DNA (Yu *et al.*, 1974). The same method can be used here for poly(Lys^{40},Ala^{60})-DNA, though light scattering during melting makes some contribution to

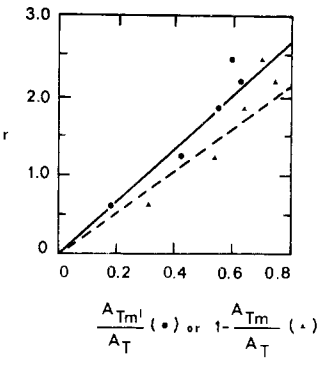

FIGURE 4: Linear plots of eq 1 and 2.

the hyperchromicity change in this system. Because of the residual hyperchromicity between the two melting bands at T_m and T_m', both eq 1 and 2 are used, where eq 2, but not

$$r = \beta A_{T_m'}/A_T \quad (1)$$

and

$$r = \beta[1 - (A_{T_m}/A_T)] \quad (2)$$

eq 1, includes the residual melting between the two melting bands as part of the melting of copolypeptide-bound base pairs. In these equations, β represents the amino acid residues per nucleotide in copolypeptide-bound regions, A_{T_m} and $A_{T_m'}$ the areas under melting bands of T_m and T_m', respectively, and A_T the total area under the curve, which is equal to h_{max}. The linear plots of these two equations are given in Figure 4. The β value obtained from eq 1 is 3.2, while that from eq 2 is 2.7 amino acid residues per nucleotide, or an average of about 3.0 amino acid residues per nucleotide in copolypeptide-bound regions. Since lysine comprises only 40% of the residues in the copolypeptide, this means there should be approximately 1.2 lysine per nucleotide in bound regions.

As will be shown later, the negative CD near 220 nm of poly(Lys^{40},Ala^{60}) is nearly doubled when it interacts with DNA. If every added copolypeptide molecule is bound to DNA in the same way, one would expect a linear relationship between its CD ($\Delta\epsilon_{220}$, for example) and r, the input ratio of copolypeptide to DNA. One would also expect the CD change induced in DNA by copolypeptide binding, changes of $\Delta\epsilon_{278}$, to be proportional to the r value of the complex. Such expectations are shown to be valid in Figure

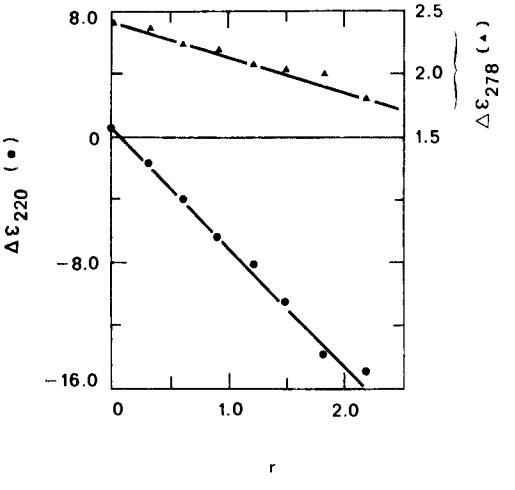

FIGURE 5: Linear dependence of CD of the complex on its r value.

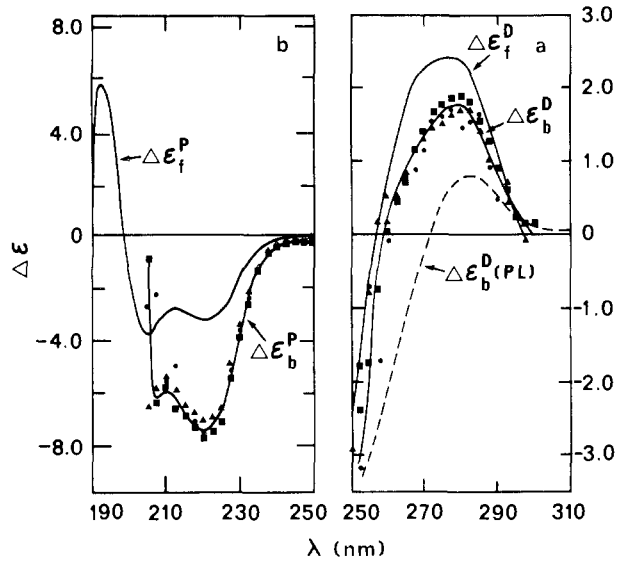

FIGURE 6: (a) Calculated CD spectrum $\Delta\epsilon_b^D$, for DNA bound by poly(Lys^{40},Ala^{60}). $\Delta\epsilon_b^D$ was calculated from eq 3 using complexes with r = 0.6 (●), 1.2 (▲), and 1.8 (■). Also included is $\Delta\epsilon_f^D$ for free DNA and $\Delta\epsilon_b^D$ for polylysine-bound DNA (Chang *et al.*, 1973). (b) Calculated CD spectrum $\Delta\epsilon_b^P$, for poly(Lys^{40},Ala^{60}) bound by DNA. $\Delta\epsilon_b^P$ was calculated from eq 5 using complexes with r = 0.6 (●), 1.2 (▲), and 1.8 (■). Also included is $\Delta\epsilon_f^P$ for free poly(Lys^{40},Ala^{60}).

5. Because of this, it is possible to determine the CD spectrum of both bound copolypeptide and bound DNA.

Above 250 nm, there is no CD contribution from copolypeptide. The CD of a complex in this region, therefore, is a sum of the CD from free and copolypeptide-bound base pairs. If F is the fraction of base pairs bound by copolypeptide in the complex, and $\Delta\epsilon_m$, $\Delta\epsilon_f^D$, and $\Delta\epsilon_b^D$ the measured CD of the complex and the CD of free and bound base pairs, respectively, eq 3 can be used. This equation has been

$$\Delta\epsilon_m = (1 - F)\Delta\epsilon_f^D + F\Delta\epsilon_b^D \quad (3)$$

used before for polylysine-DNA (Chang *et al.*, 1973), protamine-DNA (Yu and Li, 1973), and polyarginine-DNA (Yu *et al.*, 1974). F for the complex can be calculated from

$$F = r/\beta \quad (4)$$

where β, as defined earlier, is the ratio of amino acid residue to nucleotide in copolypeptide-bound regions. With the aid of β = 3.0, as determined by thermal denaturation (Figure 4), $\Delta\epsilon_b^D$ of copolypeptide-bound base pairs from three complexes of different r values were calculated from eq 3. As shown in Figure 6a, they do agree with one another. The characteristics of the CD are λ_{max} 279 nm for the peak, λ_c 259 nm for the crossover, and $\Delta\epsilon_{279}$ = 1.8. Also included in Figure 6a is $\Delta\epsilon_b^D$ of polylysine-bound base pairs in direct-mixed polylysine-DNA complex. It is seen that the conformational change in bound DNA induced by copolypeptide, as judged by CD, is similar to that induced by polylysine, but is significantly smaller in amplitude. The shift in λ_c from 256.5 nm in $\Delta\epsilon_f^D$ to 259 nm in $\Delta\epsilon_b^D$ in the case of copolypeptide is much smaller than its shift from 256.5 nm in $\Delta\epsilon_f^D$ to 272 nm in $\Delta\epsilon_b^D$ for polylysine. It should be noted that, in chromatin, histone binding on DNA also causes a great reduction in the amplitude at λ_{max} but only a small shift for λ_c. Perhaps this similarity in effect is due to the presence of α helix in bound proteins, in poly(Lys^{40},Ala^{60}) in the present system, and in histones in chromatin.

Below 250 nm, both DNA and copolypeptide contribute to the measured CD. There is a possibility that the CD of DNA in this region might be affected by the binding of copolypeptide. However, this change is probably small, as is the case for DNA in the presence of salt (Tunis-Schneider and Maestre, 1970; Li *et al.*, 1971; Ivanov *et al.*, 1973), or for DNA in polylysine-DNA complexes (Chang *et al.*, 1973). Further, the CD of DNA in this region is relatively small compared to that of copolypeptide, as shown in Figure 3. Therefore, as an approximation, it is assumed that the CD change in DNA below 250 nm caused by the binding of copolypeptide is negligibly small compared to that of the copolypeptide alone. It is then possible to use the following equation to estimate the CD of bound copolypeptide in the complex

$$\Delta\epsilon_m = \Delta\epsilon_f^D + r\Delta\epsilon_b^P \quad (5)$$

where $\Delta\epsilon_m$ and $\Delta\epsilon_f^D$ are as defined earlier in eq (3) and $\Delta\epsilon_b^P$ is the CD of bound protein in the complex. The calculated $\Delta\epsilon_b^P$ from three complexes of different ratios are shown in Figure 6b to agree with one another. Also included in Figure 6b is the CD spectrum, $\Delta\epsilon_f^P$, of free copolypeptide. This CD has double negative peaks at 222 and 205 nm and a positive peak at 193 nm which indicates the existence of α helix (Holzwarth and Doty, 1965; Greenfield and Fasman, 1969). When copolypeptide is bound, the amplitude of its CD is nearly doubled and the shape is changed to a major negative peak at 220 nm with only a shoulder at 207 nm. As the helical copolypeptide is bound to DNA, the enhancement in the amplitude can be interpreted possibly as resulting either from a distorted α helix or from a greater content of α helix when the copolypeptide is bound to DNA, or, more likely, from both. An increase in α-helical content is not impossible, because charge neutralization of lysine by phosphate could possibly stabilize more α helix in bound copolypeptide.

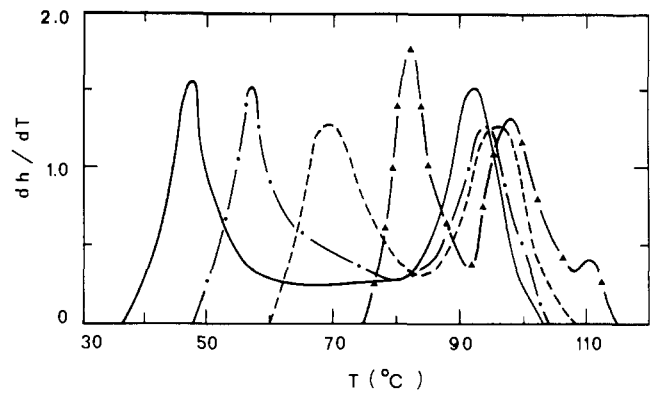

FIGURE 7: Derivative melting profiles of poly(Lys^{40},Ala^{60})-DNA complex at various ionic strengths, r = 1.2. NaCl = 0.00 M (—), 0.002 M (- · -), 0.01 M (- - -), and 0.06 M (- ▲ -).

Effect of Ionic Strength on Poly(Lys^{40},Ala^{60})-DNA Complex. As shown earlier from Figure 4, there are 1.2 lysines per nucleotide in the bound regions, which means there are more positive charges present than required to neutralize the phosphates on DNA. The fact that the T_m' of copolypeptide-bound regions (92–94°) is lower than for those bound by polylysine (99–101°) (Li *et al.*, 1973) can be due either to destabilization of DNA by hydrophobic alanine or to inadequate charge neutralization of the phosphates. Insufficient charge neutralization could occur if the α helix in the copolypeptide prevented lysine from close interaction with phosphates on DNA through a steric factor. In order to investigate this possibility, a copolypeptide–DNA complex with r = 1.2 was dialyzed in EDTA buffers of various

NaCl concentrations. The results are shown in Figure 7. As expected, the T_m of free base pairs is raised significantly at higher ionic strength; the T_m' of copolypeptide-bound base pairs is also shifted to a slightly higher temperature, which is contrary to results obtained with direct-mixed polylysine-DNA (Li *et al.*, 1974), protamine-DNA (Yu and Li, 1973), and polyarginine-DNA complexes (Epstein *et al.*, 1974), where the T_m' is independent of ionic strength. In poly(Lys^{40},Ala^{60})-DNA complexes, at higher ionic strength (0.06 M NaCl), an additional melting band appears at 100°, which becomes more significant at 0.1 M NaCl or higher. Light scattering is probably increased at this temperature, and this, in turn, contributes to the hyperchromicity at 260 nm. Therefore the analysis of ionic strength dependence of T_m and T_m' is limited to NaCl ≤0.06 M.

Equations 6 and 7 have been used previously for describ-

$$T_m = T_m^0 + a \log Na^+ \quad (6)$$

$$T_m' = (T_m')^0 + a' \log Na^+ \quad (7)$$

ing the effect of ionic strength on T_m and T_m' (Yu and Li, 1973). These equations are essentially the same as those used with pure DNA (Dove and Davidson, 1962; Schildkraut and Lifson, 1965). The plots of both equations (not shown here) yield $T_m^0 = 102°$, $(T_m')^0 = 100°$, $a = 17°$, and $a' = 2.7°$. Though the theory behind eq 7 for protein-bound base pairs has not yet been developed, the concept of electrostatic shielding by NaCl and its effect on the melting temperature of pure DNA (Dove and Davidson, 1962; Schildkraut and Lifson, 1965; Record, 1967; Manning, 1972) are used here to gain insight into the electrostatic shielding of DNA by bound proteins in a nucleoprotein (Li and Bonner, 1971; Li *et al.*, 1974). The plotted results show $a' \ll a$, which implies that copolypeptide-bound base pairs are much more electrostatically shielded than are free base pairs, but this shielding is not as great as in protamine-DNA (Yu and Li, 1973) or polylysine-DNA complexes (Li *et al.*, 1974) where a' is zero. In addition, $(T_m')^0$ is approximately equal to T_m^0, which may imply further that the presence of alanine in the protein does not adversely affect the thermal stability of DNA. It should be noted that the tyrosine residues in poly(Lys^{50},Tyr^{50}) also do not destabilize DNA in thermal denaturation (Santella and Li, 1974). In EDTA buffer, the T_m of this complex is slightly lower than the $(T_m')^0$, and also lower than the T_m' of polylysine-DNA. This may be due to a lack of full charge neutralization on the phosphate lattice caused by the α-helical structure of the protein.

The effect of ionic strength on the CD spectrum of the complex is still more striking (Figure 8). While the binding of poly(Lys^{40},Ala^{60}) to DNA in EDTA buffer without NaCl causes a slight red shift for the positive CD band of DNA near 275 nm, the transfer of this complex to another environment of higher ionic strength causes an opposite and much more pronounced CD effect. In addition to marked enhancement of the positive band and a blue shift of its peak from 278 to 260 nm, a new negative band at 295 nm appears. The positive peak at 260 nm is very similar to that of DNA dissolved in a solvent mixture of water-ethanol or water-dioxane at low water content (Ivanov *et al.*, 1973), as well as to theoretically calculated CD spectrum of A form DNA (Johnson and Tinoco, 1969; Bloomfield *et al.*, 1974); it is also very similar to that of the A-form double-stranded RNA (Samejima *et al.*, 1968; Wells and Yang, 1974). The negative peak of the copolypeptide-DNA complex at 295 nm, however, is much more pronounced than in other systems with A-type CD spectra.

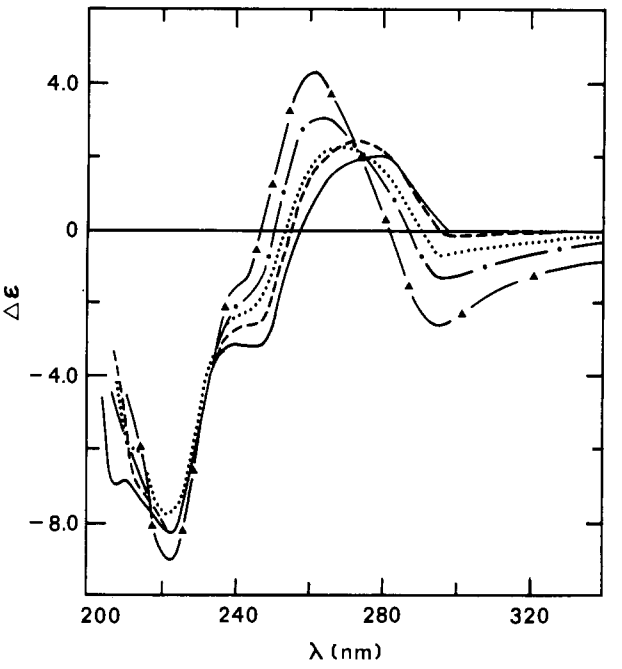

FIGURE 8: CD spectra of poly(Lys^{40},Ala^{60})-DNA complex at various ionic strengths, r = 1.2. NaCl = 0.00 M (—), 0.05 M (- - -), 0.10 M (· ··), 0.15 M (- · -), and 0.20 M (- ▲ -).

From Figure 8, it can be seen that near 220 nm the CD spectrum is insensitive to changes in NaCl. This implies either that $\Delta\epsilon_{220}$ of both copolypeptide and DNA is not affected, or that the changes for copolypeptide and DNA cancel each other in the measured $\Delta\epsilon_{220}$. The former reasoning is probably more accurate, because, in the B to A transition of pure DNA, the change in $\Delta\epsilon_{220}$ is small compared to that of $\Delta\epsilon_{260}$ (Ivanov *et al.*, 1973) and is even smaller compared to $\Delta\epsilon_{220}$ of the bound protein. Therefore, it is likely that the secondary structure of the bound copolypeptide is not significantly altered, while that of DNA measured at 260 nm is greatly distorted when the complex is transferred from a very low to an intermediate ionic environment. At the intermediate salt concentration in which this distortion first becomes significant (0.1-0.2 M NaCl) thermal denaturation studies are not feasible due to greatly increased light scattering and precipitation at higher temperatures.

The transition to A structure depends not only upon ionic strength but also upon the coverage of copolypeptide on DNA as shown in Figure 9. The A-type CD spectrum above 250 nm becomes more pronounced as more copolypeptide is bound to DNA. Figure 10 further shows that, after dialysis into 0.2 M NaCl, $\Delta\epsilon_{220}$ still depends linearly upon the r value of the complex, as was shown to be true for copolymer-DNA complexes in EDTA buffer without salt (Figure 5). This confirms the earlier suggestion that the secondary structure of bound protein is not significantly changed by the presence of NaCl in the solution. There is a very different, nonlinear dependence of the DNA CD on r value of the complex at 260 and 295 nm. Apparently at these wavelengths, the NaCl effect on the DNA structure of the complex cannot be simply decomposed into two independent components, with one type of salt effect on free DNA regions and another on copolypeptide-bound regions. The effect of NaCl on the DNA CD of the complex above 250 nm seems to be much more complicated.

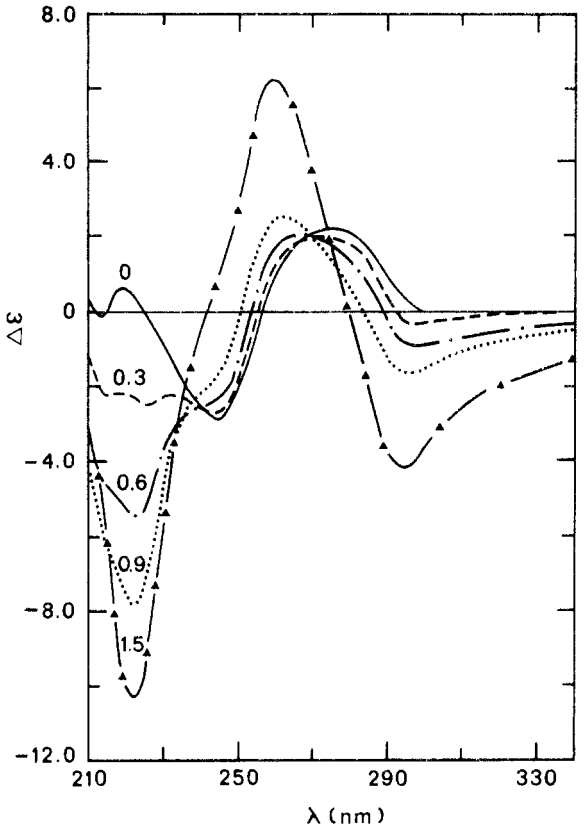

FIGURE 9: CD spectra of poly(Lys^{40},Ala^{60})–DNA complexes in 0.2 M NaCl. r of each complex is indicated.

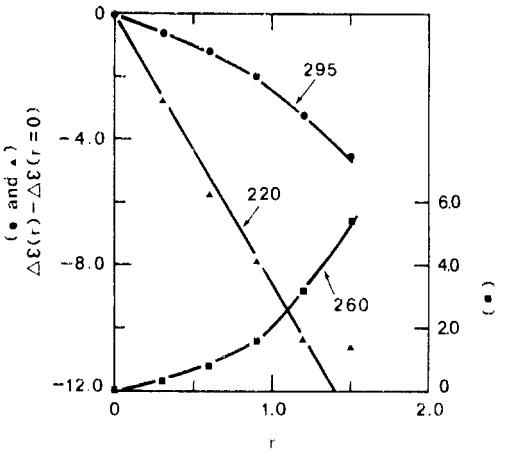

FIGURE 10: Dependence of CD changes of poly(Lys^{40},Ala^{60})–DNA complex on its r value in 0.2 M NaCl.

If the CD changes at 260 nm or 290 nm are plotted against r^2, rather than r, a linear dependence can be demonstrated. Such a dependence on r of an order higher than one implies either an intermolecular interaction among complex molecules or a cooperative intramolecular structural distortion within the complex molecule. Dependence of the CD change in DNA on complex concentration in 0.2 M NaCl was tested by measuring the CD spectra of a complex at various dilutions up to tenfold for periods of time extending up to 20 hr. In no case did dilution change the shape of the CD spectrum, and the amplitude at 260 or 295 nm was simply proportional to the concentration. This experiment may exclude the possibility that the transition to an A-type CD in the presence of salt is due to a reversible intermolecular interaction, although an irreversible interaction among the complex molecules as a cause of this transition still cannot be ruled out. An alternative suggestion for the dependence of CD change above 250 nm upon r^2 rather than r is a cooperative structural distortion of DNA molecules bound by this copolymer. Such a possibility is not unreasonable, since, with respect to base rotation, tilting, and stacking, the change from B to A form DNA is of much greater magnitude than is the transition to C structure (Arnott *et al.*, 1967; Bloomfield *et al.*, 1974). The transition B → A may be energetically less favorable than a transition from B to C form, such that maintenance of a copolypeptide-bound region in A conformation with the neighboring free regions in B structure would be unlikely. The presence of another copolypeptide in the neighborhood of this bound region might provide additional energy to permit cooperative action within the DNA molecule and cause a conformational change toward A structure.

Discussion

Studies of protein–DNA interaction are much more complicated than those of pure DNA or proteins alone, because the individual properties of DNA and proteins have to be considered simultaneously. Three major aspects of interaction are commonly considered when a protein is bound to DNA: selectivity of a DNA sequence for binding (Jacob and Monod, 1961; Gilbert and Muller-Hill, 1967; Teng *et al.*, 1971; Leng and Felsenfeld, 1966), thermal stabilization of bound base pairs (Tsuboi *et al.*, 1966; Olins *et al.*, 1967,1968; Kawashima *et al.*, 1969; Shih and Bonner, 1970; Ansevin and Brown, 1971; Li and Bonner, 1971; Li, 1972,1973), and structural effects, both on protein (Li *et al.*, 1971; Shih and Fasman, 1971) and on DNA (Fasman *et al.*, 1970; Shih and Fasman, 1970,1971; Simpson and Sober, 1970; Permogorov *et al.*, 1970; Johnson *et al.*, 1972; Chang *et al.*, 1973). The last two aspects are dealt with extensively in this report for the model protein, poly(Lys^{40}, Ala^{60}).

In nucleohistones, the melting temperatures of histone-bound regions are generally lower than those found in basic homopolymers such as polylysine and polyarginine. The most obvious explanations for the lowering of these melting temperatures are either (a) that the presence of nonbasic amino acid residues destabilizes DNA base pairs or (b) that phosphates on the DNA are not fully neutralized by direct interaction with cations on the proteins. The results in Figure 7 and the linear plots of eq 6 and 7 seem to indicate that alanine itself does not destabilize DNA to a detectable level. Instead, alanine in the free copolypeptide simply causes it to form a rigid α helix, which, when bound to DNA, prohibits full ionic interaction between lysine and phosphate, and this leads to a lower T_m'. The fact that melting temperatures for histone-bound base pairs in nucleohistones are lower than those of polylysine–DNA, despite roughly equal proportion of basic amino acid residues and phosphates in histone-bound regions of chromatin (Li, 1973; Li *et al.*, 1973), may be explained in a similar way.

Although it has been reported previously that the CD of histone IV (f2al) is distorted when it is complexed with DNA in reconstituted nucleohistone (Li *et al.*, 1971; Shih and Fasman, 1971), with reconstitution, the cause of the change is more complicated than with direct mixing, because during salt gradient dialysis it is not known under what specific condition the histone is complexed to DNA. In addition, in free state, histone IV has not only α helix,

but β sheet and random coil as well (Li *et al.*, 1972). The simpler copolypeptide-DNA system under study has a great advantage in that poly(Lys^{40},Ala^{60}) has an α-helical structure in the free state, and by using the method of direct mixing the condition of interaction can be controlled.

The enhanced but distorted CD spectrum of the bound copolymer shown in Figure 6B could be explained in two ways. Perhaps there is a strong interaction between the amide of the copolypeptide and the DNA, or there may be a distortion of the regular α helix of the copolymer when it is bound. Because of these possibilities, it is necessary to be cautious in analyzing the protein CD spectrum of a protein-DNA complex. Simply using as reference the spectra of free proteins in α-helix, β-sheet, and random coil conformations, without considering the possible distortion of its secondary structure by binding, could be very misleading.

Theoretically the optical properties of DNA have been studied extensively by Tinoco and his collaborators (Tinoco, 1968; Johnson and Tinoco, 1969), and experimentally by many laboratories. In general, three different types of CD correlate with the three conformations of DNA described as, A, B, and C forms (Arnott *et al.*, 1967; Bloomfield *et al.*, 1974). Depending upon the proteins and the manner of making the complexes, when a protein is bound to B-form DNA, the conformation of DNA, as judged by CD, may be unchanged (Olins, 1969; Wagner, 1970; Li *et al.*, 1971; Shih and Fasman, 1971), distorted toward C form (Chang *et al.*, 1973; Yu and Li, 1973; Yu *et al.*, 1974; Leffak *et al.*, 1974), distorted toward A form (Shih and Fasman, 1971; Adler *et al.*, 1974), or toward another structure exhibiting a huge negative CD near 270 nm (Shapiro *et al.*, 1969; Haynes *et al.*, 1970; Fasman *et al.*, 1970; Sponar and Fric; 1972, Carroll, 1972, Jordan *et al.*, 1972).

Transitions from B conformation, B $\rightarrow$ C and B $\rightarrow$ A, belong to different categories with respect to base rotation, tilting, and stacking (Bloomfield *et al.*, 1974). Earlier reports on the CD of nucleoproteins always dealt exclusively with transitions from B conformation: B toward C, B toward A, or B toward other structures. The evidence in Figure 8 demonstrates a transition of B toward C followed by a further transition toward A. The B $\rightarrow$ C transition occurs when DNA is complexed with the copolypeptide in EDTA buffer; the further transition toward A is then induced as the initially distorted complex is transferred from EDTA buffer to an EDTA buffer containing NaCl.

Since the CD of the bound copolypeptide is not significantly changed in salt, its secondary structure presumably is not changed either. However, charge distribution along the complex molecule changes markedly from free to copolypeptide-bound DNA regions. The presence of significant concentrations of Na^+ and Cl^- ions along the complex molecule could apply different forces to bound and free regions of DNA, and cause base tilting. The base tilting of A form produces a 20° angle between the base pairs and the plane perpendicular to the helical axis of DNA, which represents the greatest tilt among the three common structures (Bloomfield *et al.*, 1974). In the presence of salt, it is also possible that the tertiary structure of bound copolypeptide is changed, and that hydrophobic interaction between DNA base pairs and alanine residues, or between the alanine residues themselves, would become more important. Since the bases in A conformation are more exposed to the outside than in B or C structure, hydrophobic interaction between these bases and alanine residues might be favored in the A structure.

In summary, the results reported in this communication indicate that (a) poly(Lys^{40},Ala^{60}) in solution has an α-helical structure which can interact directly with DNA, causing a distortion of its structure, (b) the presence of alanine reduces the B $\rightarrow$ C transition of DNA induced by polylysine, (c) the presence of α helix reduces the efficiency of direct charge neutralization of phosphates by lysine, so that the T_m' is lower than the corresponding melting temperature of polylysine-DNA, and (d) the intermediate DNA conformation, B $\rightarrow$ C, of the complex can undergo a further C $\rightarrow$ A transition when it is transferred from very low to intermediate ionic strength. This research demonstrates the potential of a new direction of research emphasizing the role of individual amino acid residues and of secondary and tertiary structures in a protein as it interacts with DNA. This report also suggests model systems with which to study DNA structures and the factors which govern them.

References

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

Ansevin, A. T., and Brown, B. W. (1971), *Biochemistry 10,* 1133.

Arnott, S., Wilkins, M. H. F., Fuller, W., and Langridge, R. (1967), *J. Mol. Biol. 27,* 535.

Bloomfield, V. A., Crothers, D. M., and Tinoco, I., Jr. (1974), Physical Chemistry of Nucleic Acids, New York, N. Y., Harper and Row.

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

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

Clark, R. J., and Felsenfeld, G. (1971), *Nature (London), New Biol. 229,* 101.

Dove, W. F., and Davidson, N. (1962), *J. Mol. Biol. 5,* 467.

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

Fasman, G. D., Bodenheimer, E., and Resce, A. (1965), *J. Biol. Chem. 241,* 917.

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

Gilbert, W., and Muller-Hill, B. (1967), *Proc. Nat. Acad. Sci. U. S. 58,* 2415.

Greenfield, N., and Fasman, G. D. (1969), *Biochemistry 8,* 4108.

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

Holzwarth, G., and Doty, P. (1965), *J. Amer. Chem. Soc. 87,* 218.

Ivanov, V. I., Minchenkova, L. E., Schyolkina, A. K., and Poletaev, A. I. (1973), *Biopolymers 12,* 89.

Jacob, F., and Monod, J. (1961), *J. Mol. Biol. 3,* 318.

Johnson, R. S., Chan, A., and Hanlon, S. (1972), *Biochemistry 11,* 4348.

Johnson, W. C., Jr., and Tinoco, I. Jr. (1969), *Biopolymers 7,* 727.

Jordan, C. F., Lerman, L. S., and Venable, J. H., Jr. (1972), *Nature (London), New Biol. 236,* 67.

Kawashima, S., Inoue, S., and Ando, T. (1969), *Biochim. Biophys. Acta 186,* 145.

Leach, S. J., and Scheraga, H. A. (1960), *J. Amer. Chem. Soc. 82,* 4790.

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

Leng, M., and Felsenfeld, G. (1966), *Proc. Nat. Acad. Sci. U. S. 56,* 1325.
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. (1974), *Biopolymers 13,* 1681.
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.
Li, H. J., Wickett, R., Craig, A. M., and Isenberg, I. (1972), *Biopolymers 11,* 375.
Manning, G. S. (1972), *Biopolymers 11,* 937.
Morita, K., Simons, E. R., and Blout, E. R. (1967), *Biopolymers 5,* 260.
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.
Olins, D. E., Olins, A. L., and von Hippel, P. H. (1968), *J. Mol. Biol. 33,* 265.
Permogorov, U., Debabov, I. U. G., Sladkova, I. A., and Rebentish, B. A. (1970), *Biochim. Biophys. Acta 199,* 556.
Record, M. T., Jr. (1967), *Biopolymers 5,* 975.
Samejima, T., Hashizume, H., Imahori, K., Fuzii, I., and Miura, K. (1968), *J. Mol. Biol. 34,* 39.
Santella, R., and Li, H. J. (1974), *Biopolymers* (in press).
Schildkraut, C., and Lifson, S. (1965), *Biopolymers 3,* 195.
Shapiro, J. T., Leng, M., and Felsenfeld, G. (1969), *Biochemistry 8,* 3219.
Shih, T. Y., and Bonner, J. (1970), *J. Mol. Biol. 48,* 469.
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.
Spies, J. R. (1957), *Methods Enzymol, 3,* 467.
Sponar, J., and Fric, I. (1972), *Biopolymers 11,* 2317.
Sponar, J., Stokrova, S., Koruna, I., and Blaha, K. (1974), *Collect. Czech. Chem. Commun. 39,* 1625.
Stein, G. S., Spelsberg, T. C., and Kleinsmith, L. J. (1974), *Science 183,* 817.
Teng, C. S., Teng, C. T., and Allfrey, V. G. (1971), *J. Biol. Chem. 246,* 3597.
Tinoco, I., Jr. (1968), *J. Chim. Phys. Physicochim. Biol. 65,* 91.
Tunis-Schneider, M. J. B., and Maestre, M. F. (1970), *J. Mol. Biol. 52,* 521.
Tsuboi, M., Matsuo, K., and Ts'o, P. O. P. (1966), *J. Mol. Biol. 15,* 256.
Wagner, T. E. (1970), *Nature (London) 227,* 65.
Wells, B. D., and Yang, J. T. (1974), *Biochemistry 13,* 1317.
Yu, S. S., Epstein, P., and Li, H. J. (1974), *Biochemistry 13,* 3713.
Yu, S. S., and Li, H. J. (1973), *Biopolymers 12,* 2777.