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Interactions between Model Proteins and Deoxyribonucleic Acids

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ABSTRACT: Interactions between DNA and model proteins, poly(L-Lys^{m}L-Ala^n), where $m + n = 100\%$, have been investigated using thermal denaturation and circular dichroism (CD). All complexes of DNA with these proteins precipitate in a small range of input ratios, protein to DNA, with the midpoints of all precipitation curves close to a 1:1 ratio of lysine to phosphate. The melting temperature of model protein-bound DNA regions decreases slightly as the alanine content of the model protein is increased, which can be explained as a result of insufficient charge neutralization of phosphates by lysine residues in the model proteins. In the free state, these model proteins possess varying amounts of α helix, random coil, or a mixture of these two, depending upon the relative lysine/alanine content. When bound to DNA, the CD of the complex shows a substantial increase in α-helical structure for those model proteins with 40–60% alanine, while there is no significant change in α-helical structure when the percent alanine is either substantially higher or lower (i.e., 81 or 19% alanine). Only those complexes formed with model proteins having 40–60% alanine undergo a drastic transition from a B-type CD to an A-type in the presence of intermediate ionic strength (0.2 M NaCl, for example). Poly(Lys^{19}Ala^{81})·DNA complexes show a slight transition toward A-type CD at 0.4 M NaCl or higher. Apparently other factors, in addition to alanine and α-helical content, must be responsible for this B $\rightarrow$ A transition. At the other extreme of lysine/alanine ratio, with high lysine content, poly(Lys^{81}Ala^{19}) or polylysine, the presence of NaCl produces a B $\rightarrow \Psi$ transition. The possible significance of these differences in response to the binding of these model proteins is discussed.

Protein-DNA interactions relate closely at the molecular level to both chromosome structure and gene regulation. Interactions between DNA and proteins, such as histones and the lactose repressor, have been studied in many laboratories (Hnilica, 1972; Johnson et al., 1974; Von Hippel and McGhee, 1972). In order to understand these interactions, model proteins, in which such parameters as amino acid composition, sequence, and secondary structure can be controlled, have been investigated (Friedman and Ts'o, 1971; Sponar et al., 1974; Santella and Li, 1974; Pinkston and Li, 1974; Mandel and Fasman, 1974; Stokrova et al., 1975; Santella and Li, 1975).

Previously we reported the results of thermal denaturation and circular dichroism (CD) studies on poly(Lys^{40}Ala^{60})-DNA complexes (Pinkston and Li, 1974). Poly(Lys^{40}Ala^{60}) contains some degree of α-helical structure in the free state. When it is complexed with DNA, its α-helical content is markedly increased while the DNA conformation moves from B slightly toward C form. Change in the ionic environment of the complex by the addition of NaCl induces a further structural transition of the DNA from partial C form toward A conformation. In order to probe more extensively the effects of complex formation on the secondary structures of both model proteins and DNA, as well as the structural effects due to changes in ionic conditions, interactions between DNA and a series of related model proteins, poly(Lys^{m}Ala^n), where $m + n = 100\%$, were studied by means of thermal denaturation and CD. Variation in the lysine and alanine content of the

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Table I: Midpoints of Precipitation Curves of Poly(Lys^{m}Ala^n)·DNA Complexes.

Copolypeptide m/n	19/81	40/60	48/52	57/43	67/33	81/19
r_{mid}(amino acid/nucleotide)	4.40	2.33	1.93	1.50	1.42	1.23
r_{mid}(lysine/nucleotide)	0.84	0.96	0.93	0.86	0.95	1.00

copolymer causes the model protein to assume a conformation containing more or less random coil or α helix. These studies therefore provide model systems for investigation of the roles played both by alanine as a hydrophobic amino acid residue and by α-helical structure in a protein when such protein interacts with DNA.

Materials and Methods

Random poly(Lys^{m}Ala^n), copolymers of m% L-lysine and n% L-alanine where $m + n = 100$%, were synthesized using the *N*-carboxyanhydrides of ε-carbobenzyloxy-L-lysine and -L-alanine (Miles Laboratories) according to the methods of Fasman et al. (1965) and Morita et al. (1967). The amino acid compositions of the copolypeptides were determined by an amino acid analyzer. The composition of each was very close to the input ratio before polymerization. Concentrations of stock solutions of copolypeptide, poly(Lys^{m}Ala^n), were determined by the ninhydrin method (Spies, 1957), using a mixture of m% L-lysine and n% L-alanine as the standard.

Other materials and methods used in this report are essentially the same as those reported earlier (Pinkston and Li, 1974). In brief, the complexes were made by direct mixing with the slow addition of copolypeptide to DNA in 2.5×10^{-4} M EDTA, pH 8.0 (EDTA buffer). Portions of the complexes are then dialyzed to EDTA buffer containing various NaCl concentrations for studies on the effects of ionic strength. Thermal denaturation results were reported as dh/dT, where h is the hyperchromicity at 260 nm and T is the temperature. The CD results are reported as $\Delta\epsilon = \epsilon_L - \epsilon_R$, in M^{-1} cm^{-1}. For both DNA and the complexes, M represents moles of nucleotide/liter; for free and bound copolypeptides, moles of amino acid residues/liter.

Results

Titration of DNA by Poly(Lys^{m}Ala^n). Precipitation curves for poly(Lys^{m}Ala^n)·DNA complexes made by direct mixing in EDTA buffer were measured in a manner similar to that described in an earlier report for poly(Lys^{40}Ala^{60}) (Pinkston and Li, 1974). For each copolypeptide, the complex is fully soluble until a certain input ratio, r, of copolypeptide to DNA (reported in amino acid residues/nucleotide) is reached. At that point the solution shows strong light scattering, and the complex can be removed from solution by low-speed centrifugation. The midpoints, r_{mid}, of precipitation curves for these complexes are summarized in Table I. Although the r_{mid} in amino acids/nucleotide increases progressively from 1.2 to 4.4 for copolypeptides with increasing alanine content, the midpoint in terms of lysine/nucleotide (i.e., positive/negative charge) varies irregularly between 0.84 (for 81% alanine) and 1.0 (for 81% lysine). In the case of 100% lysine (polylysine), r_{mid} also occurs at 1.0 lysine/nucleotide (Clark and Felsenfeld, 1971; Li et al., 1973). These results suggest that the binding is noncooperative and that the precipitation occurs sharply when the whole complex is electrostatically neutralized, regardless of whether the free model protein is an α helix or a random coil.

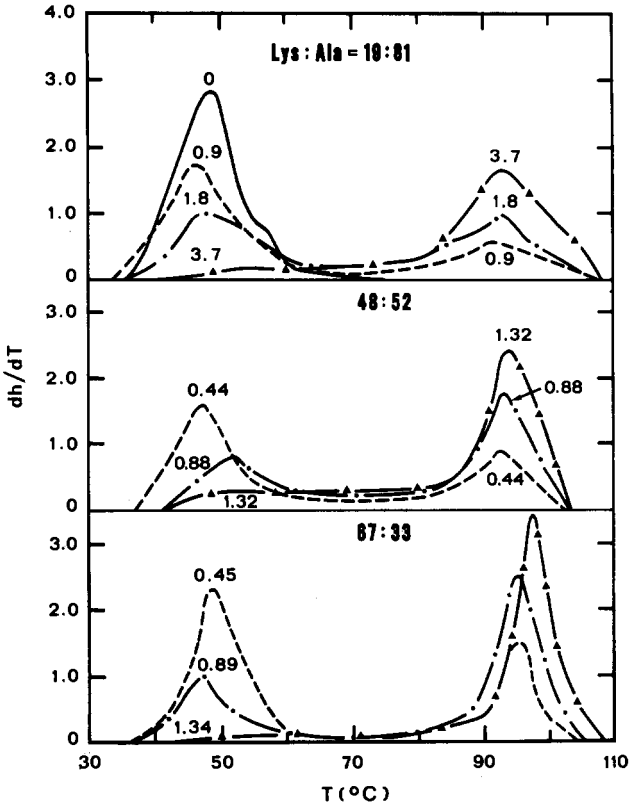

FIGURE 1: Derivative melting profiles of poly(Lys^{m}Ala^n)·DNA complexes. Both the ratio of m to n and the r value of each complex are indicated.

Thermal Denaturation of Poly(Lys^{m}Ala^n)·DNA Complexes. Figure 1 shows the derivative melting profiles of complexes made from three different copolypeptides. In all cases, biphasic melting curves are induced by the binding of copolypeptide to DNA: one phase of melting occurs about 48 °C (T_m) and another phase between 90 and 100 °C (T_m'). Although the patterns of melting are approximately the same among various copolypeptides, two distinctions can be observed in Figure 1. First, the T_m' is 91–93 °C for complexes with poly(Lys^{19}Ala^{81}), 93–94 °C with poly(Lys^{48}Ala^{52}), and 95–98 °C with poly(Lys^{67}Ala^{33}). As reported earlier, in the case of polylysine the T_m' is 99–101 °C (Li et al., 1973). The T_m' therefore gradually increases as the lysine content of the model protein is increased. Secondly, the T_m' melting band is broader for a model protein of higher alanine content. The width of the T_m' melting band at its half-height is about 15 °C for complexes with poly(Lys^{19}Ala^{81}), 10 °C with poly(Lys^{48}Ala^{52}), 7 °C with poly(Lys^{67}Ala^{33}) (Figure 1), and 5 °C with polylysine (Li et al., 1973), which seems to indicate that the melting of protein-bound DNA is less cooperative when the α-helical content of the protein is increased.

In line with our earlier report on poly(Lys^{40}Ala^{60}) (Pinkston and Li, 1974), there is residual melting between the two major melting bands of T_m and T_m' which is more pronounced for those complexes having a higher alanine content in the model protein. Analyses of melting data will then differ according to whether this residual melting, or hyperchromicity, is included in the T_m band or in the T_m' band. One or the other of the following two equations can be used, depending upon the approach chosen

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

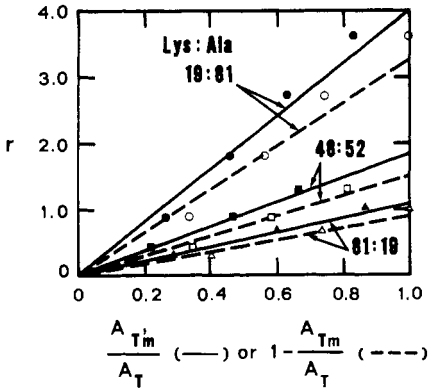

FIGURE 2: Linear plots of eq 1 and 2 for poly(Lys^mAla^n)·DNA complexes: eq 1 (—) and 2 (- - -).

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

where β represents the amino acid residues per nucleotide in copolypeptide-bound regions, A_{T_m} and $A_{T_m'}$ represent the areas under melting bands of T_m and T_m', respectively, and A_T is the total area under the curve which is equal to the maximum hyperchromicity. Plots of both equations for each complex formed from three different copolypeptides are shown in Figure 2. The β values obtained for the same complex are always greater from eq 1 than from eq 2. Averaging the β values derived from both equations, there are 3.6 amino acid residues/nucleotide for poly($Lys^{19}Ala^{81}$), 1.7 for poly($Lys^{48}Ala^{52}$), and 1.1 for poly($Lys^{81}Ala^{19}$).

Figure 3 shows the dependence of both the average β value and the T_m' of the complexes on the alanine content in the model protein. The T_m' of each model protein represents the average value obtained from complexes of various r values. In protein-bound regions the T_m' decreases linearly as the alanine content of the protein is increased. On the other hand, the β value in the protein-bound regions increases sharply when the alanine content reaches 50%. This seems to correlate with a sharp increase in α-helical content of these copolypeptides when they are bound to DNA, as is demonstrated later in Figure 7.

Circular Dichroism of Poly(Lys^mAla^n)·DNA Complexes. Figure 4 shows CD spectra of some poly(Lys^mAla^n)·DNA complexes. Complexes of poly($Lys^{19}Ala^{81}$) (Figure 4a) produce a big negative CD band at 220 nm and a shoulder at 210 nm, with the amplitude proportional to the r value of the complexes. Such CD in this wavelength region indicates a large α-helical content in the protein moiety of the complex. The effect on the DNA CD in the 275-nm region produced by the binding of poly($Lys^{19}Ala^{81}$) is, however, quite small, both in amplitude and in red shift. As the alanine content of each copolypeptide chosen for complexing is reduced, (Figure 4a–d), there is a great reduction in the protein CD near 220 nm, accompanied by an increasing effect on the DNA CD near 275 nm.

Based upon the results of thermal denaturation studies, it is possible to determine the fraction (F) of base pairs in each complex bound by model protein. By means of eq 3 it then is possible to calculate the CD ($\Delta\epsilon_b^D$) of DNA in each complex, when bound by different model proteins, using the assumption that the measured CD ($\Delta\epsilon_m$) at $\lambda > 250$ nm is a linear combination of the CD of free ($\Delta\epsilon_f^D$) and of bound DNA ($\Delta\epsilon_b^D$).

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

This assumption is based on the fact that above this wavelength there is no CD contribution from the model proteins. Equation

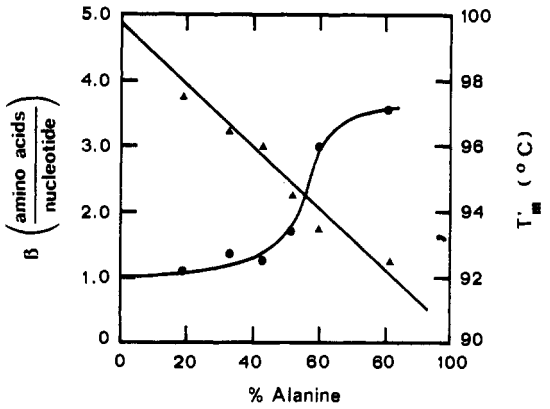

FIGURE 3: Dependence of melting temperature and β value of protein-bound regions in the complexes upon the alanine content in the model protein. Melting temperature (▲) and β value (●).

3 has been used before for polylysine·DNA (Chang et al., 1973), protamine·DNA (Yu and Li, 1973), polyarginine·DNA (Yu et al., 1974), poly($Lys^{40}Ala^{60}$)·DNA (Pinkston and Li, 1974), and poly($Lys^{58}Phe^{42}$)·DNA (Santella and Li, 1975).

Figure 5 shows the calculated CD spectra above 250 nm of complexes from three different model proteins. In each case, the calculated DNA CD spectrum, $\Delta\epsilon_b^D$, is independent of the r value of the complex. With poly($Lys^{19}Ala^{81}$), $\Delta\epsilon_b^D$ is very close to that of free DNA, $\Delta\epsilon_f^D$, having a reduction of about 10% in amplitude but no appreciable red shift in the spectrum. For poly($Lys^{57}Ala^{43}$), there is a substantial red shift in both peak and crossover as well as a reduction of 20% in amplitude at 275 nm. When the lysine content is increased to 100%, both the red shift and the reduction in amplitude are considerably greater.

Below 250 nm, both DNA and model proteins contribute to the measured CD. As discussed in an earlier paper (Pinkston and Li, 1974), it is assumed that the DNA CD is not significantly distorted by the binding of these model proteins in the lower region of the spectrum, since DNA shows little change there in the presence of salt (Tunis-Schneider and Maestre, 1970; Li et al., 1971; Ivanov et al., 1973) or bound by polylysine (Chang et al., 1973), protamine (Yu and Li, 1973), or polyarginine (Yu et al., 1974). With this assumption it is possible to calculate the CD of bound model proteins ($\Delta\epsilon_b^P$) in the complex using the following equation:

$$\Delta\epsilon_m = \Delta\epsilon_f^D + r\Delta\epsilon_b^P \quad (4)$$

Figure 6 shows the measured CD spectra of free ($\Delta\epsilon_f^P$) and the calculated spectra of bound ($\Delta\epsilon_b^P$) model proteins. In Figure 6a both spectra are shown for poly($Lys^{19}Ala^{81}$). With free copolypeptide, there are two negative peaks, at 222 and 208 nm, and a positive peak at 191 nm. Both shape and amplitude of these peaks suggest that, when free, this model protein is in completely α-helical conformation (Holzwarth and Doty, 1965; Greenfield and Fasman, 1969; Chen et al., 1972). After complex formation, the CD of bound protein ($\Delta\epsilon_b^P$) above 205 nm is approximately identical with that of the free protein ($\Delta\epsilon_f^P$); below this wavelength no reliable signal could be obtained under the experimental conditions employed.

With poly($Lys^{48}Ala^{52}$), the free model protein ($\Delta\epsilon_f^P$) has one major negative peak at 202 nm and a minor one at 222 nm. Both shape and amplitude of peaks suggest that this model protein possesses a mixture of both α-helix and random coil. When complexed with DNA, the CD of bound protein ($\Delta\epsilon_b^P$) differs greatly from that of the free protein in that a minor negative peak becomes a major peak at 222 nm, with a minor

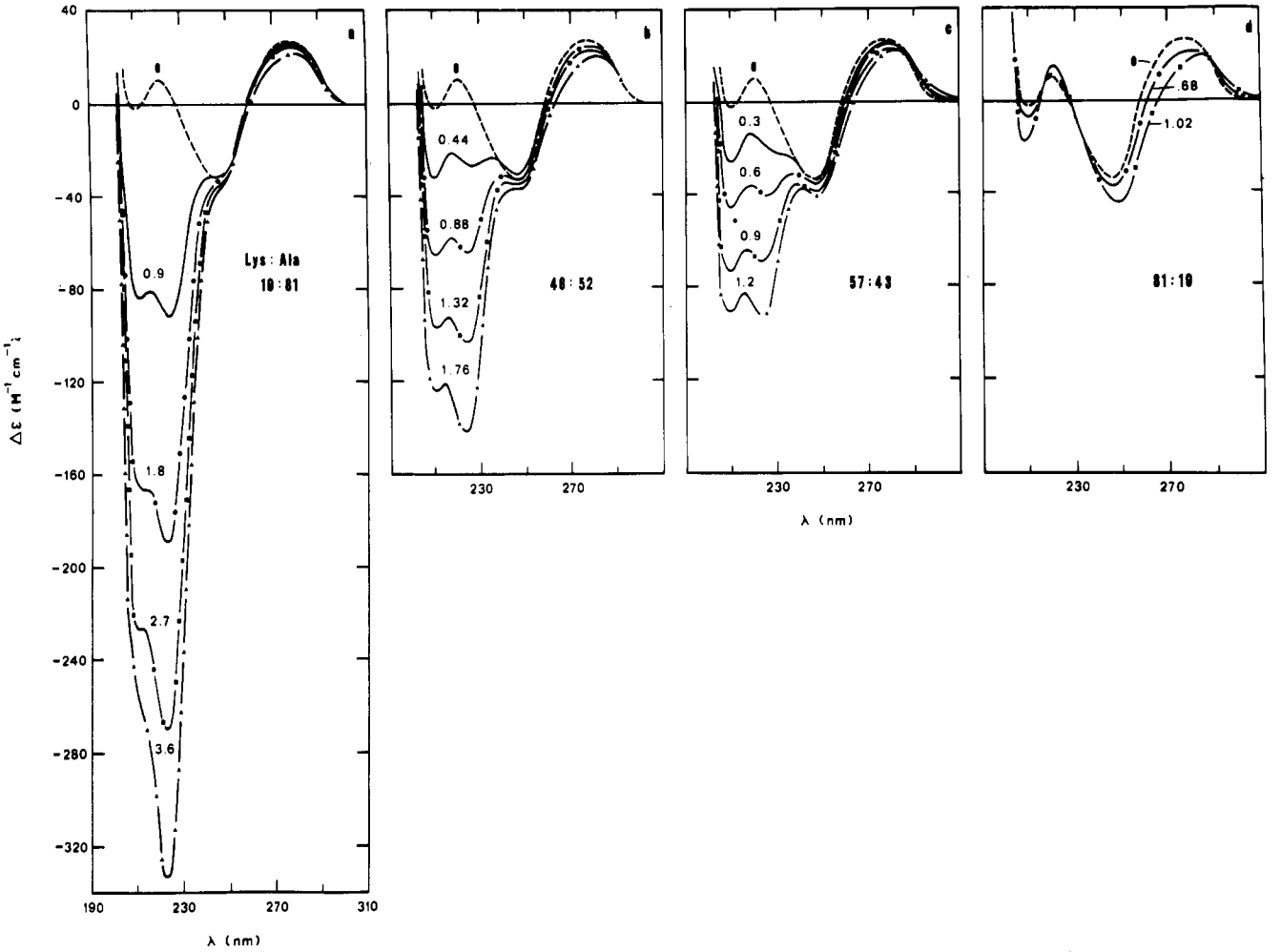

FIGURE 4: CD spectra of poly(Lys^{m}Ala^n)·DNA complexes. Both the ratio of m to n and the r value of each complex are indicated.

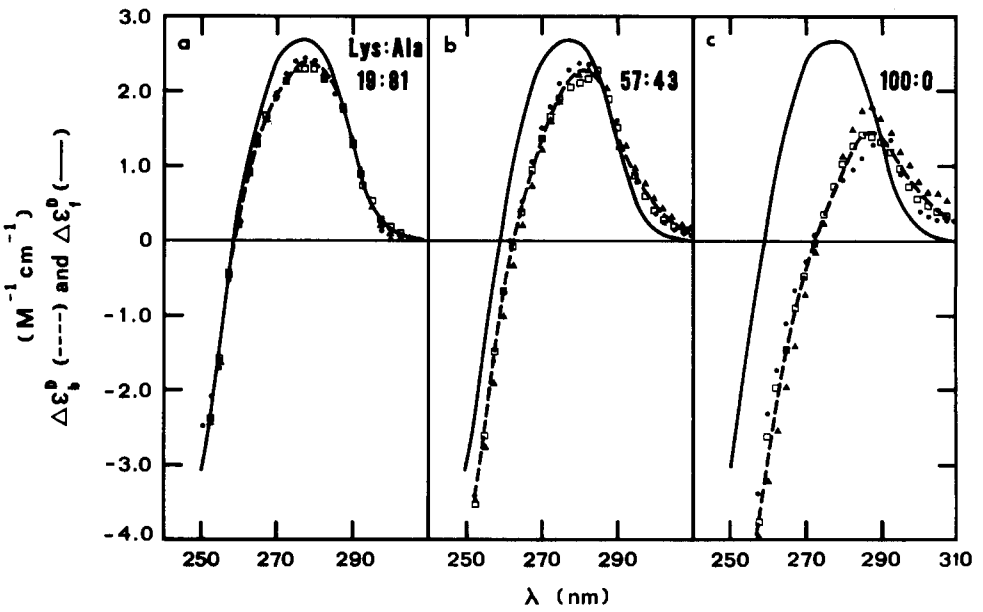

FIGURE 5: Calculated CD spectra, $\Delta\epsilon_b^D$, for DNA bound by model proteins: (a) from poly(Lys^{19}Ala^{81})·DNA complexes with r = 1.8 (●), 2.7 (□), and 3.6 (▲); (b) from poly(Lys^{57}Ala^{43})·DNA complexes with r = 0.60 (●), 0.9 (□), and 1.2 (▲); (c) polylysine·DNA complexes with r = 0.40 (●), 0.60 (□), and 0.80 (▲). Also included is $\Delta\epsilon_f^D$ for free DNA (—).

one at 210 nm. These CD changes induced by binding are similar to those reported earlier for poly(Lys^{40}Ala^{60}).

The results with poly(Lys^{57}Ala^{43}), both free and complexed, are similar to those of poly(Lys^{48}Ala^{52}), except that, in free state ($\Delta\epsilon_f^P$), the former has more random coil and less α helix than the latter (a smaller CD at 220 nm and a bigger CD at 200 nm). This is expected from its higher content of positively charged lysine residues.

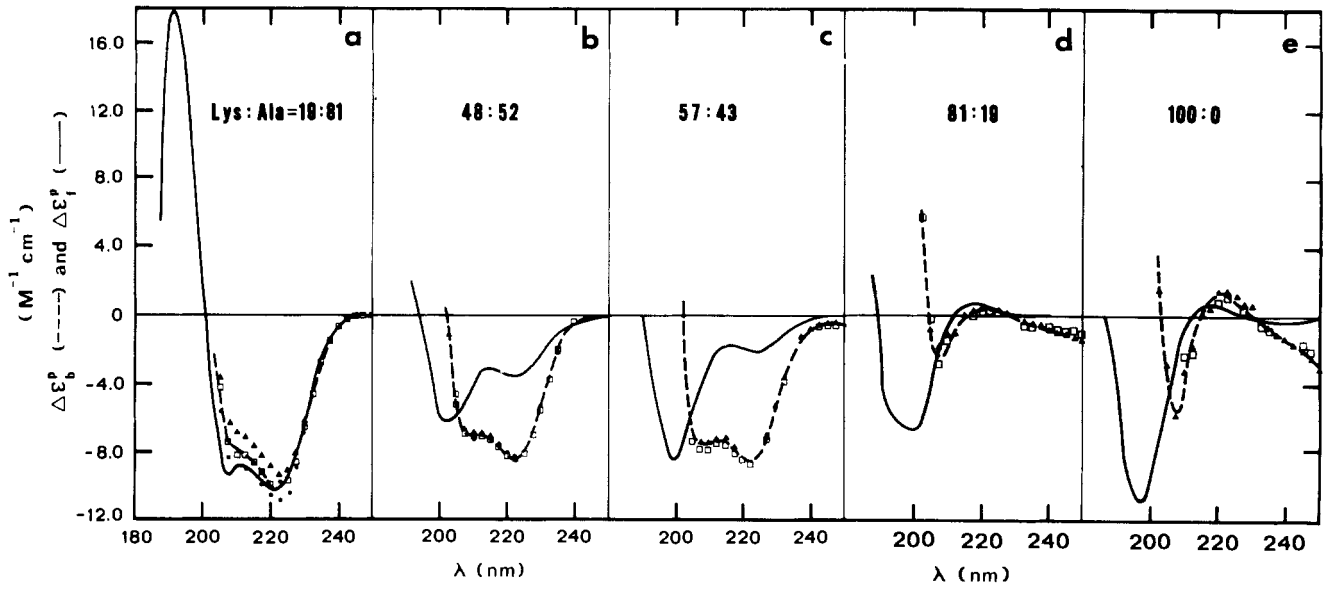

FIGURE 6: Calculated CD spectra, $\Delta\epsilon_b^P$, for poly(Lys^{m}Ala^n) bound by DNA: (a) from poly(Lys^{19}Ala^{81})-DNA complexes with r = 1.8 (●), 2.7 (□), and 3.6 (▲); (b) from poly(Lys^{48}Ala^{52})-DNA complexes with r = 1.3 (□) and 1.8 (▲); (c) from poly(Lys^{57}Ala^{43})-DNA complexes with r = 0.9 (□) and 1.2 (▲); (d) from poly(Lys^{81}Ala^{19})-DNA complexes with r = 0.68 (□) and 1.0 (▲); (e) from polylysine-DNA complexes with r = 0.60 (□) and 0.80 (▲). Also included is $\Delta\epsilon_f^P$ for free model protein (—).

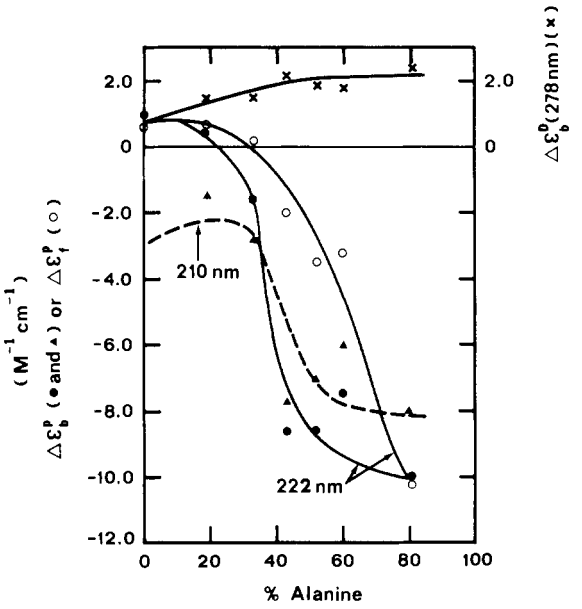

FIGURE 7: Dependence of $\Delta\epsilon_b^D$ (278 nm), $\Delta\epsilon_f^P$ (222 nm), and $\Delta\epsilon_b^P$ (222 and 210 nm) upon the alanine content in the model protein.

It is interesting to compare the CD results in Figure 6a, b, and c of this report with Figure 6a in Pinkston and Li (1974). Although the CD of free model protein ($\Delta\epsilon_f^P$) is changed substantially when the lysine content in the protein is increased from 19 to 57%, the CD of bound proteins ($\Delta\epsilon_b^P$) is not significantly changed. In other words, although the secondary structures of these model proteins in free state are very different, due to their difference in lysine or in alanine content, they do have similar secondary structure after being bound to DNA. Perhaps charge neutralization of lysine residues, effected by their binding to phosphates in DNA, stabilizes α-helical structures.

When the lysine content in the copolypeptide is increased to 81% (Figure 6d), or 100% (Figure 6e), $\Delta\epsilon_f^P$ shows a negative peak at 198–200 nm and a small positive peak at 218 nm, suggesting that these model proteins are mainly in random coil conformation (Holzwarth and Doty, 1965; Greenfield and Fasman, 1969; Chen et al., 1972). When they are complexed with DNA, the calculated CD's of bound protein are small above 215 nm. Amplitudes of resultant CD's can be greatly influenced both by the accuracy of our assumption basic to eq 4, namely, no change in the CD of DNA in this wavelength region after complexing with model proteins, and by the lower ratio of signal to noise. Nevertheless, the results clearly indicate that no α-helical structures have been induced in these complexes. In addition, the consistent upturn of the CD of these bound model proteins below 208 nm, no matter which protein is used, indicates the great effect on the CD of random coil in the complex.

Figure 7 summarizes the calculated CD results as a function of the alanine content in the copolypeptide: $\Delta\epsilon_b^D$ at 278 nm for DNA, and $\Delta\epsilon_b^P$ and $\Delta\epsilon_f^P$ at 222 and 210 nm for protein. As the alanine content is increased, the protein-bound DNA $\Delta\epsilon_b^D$ at 278 nm gradually increases toward the B-type CD observed in free DNA. With free model protein, the main decrease in $\Delta\epsilon_f^P$ at 222 nm occurs when the alanine content is greater than 40%, whereas such transition occurs at lower alanine content when the protein is bound.

Figure 8 shows the difference CD between the free and bound state of DNA or protein. When the model protein contains more alanine, the difference in DNA CD becomes smaller, as was seen before in Figure 5. The difference in protein CD, measured either at 222 nm or at 210 nm, shows a maximum at 40–50% alanine. In other words, the greatest CD change induced in protein by binding to DNA occurs for those model proteins containing 40–50% alanine.

Effect of Ionic Strength on Poly(Lys^{m}Ala^n)-DNA Complexes. Figure 9 shows the derivative melting profiles of several poly(Lys^{m}Ala^n)-DNA complexes at various ionic strengths. In all cases, a higher ionic strength in the medium raises the melting band of free DNA (T_m) to a much greater extent than it does that of bound DNA (T_m') which is affected only slightly. This was also found to be true, as reported earlier, for poly(Lys^{40}Ala^{60})-DNA complexes (Pinkston and Li, 1974). As before, the small shift in T_m' can be interpreted as due to

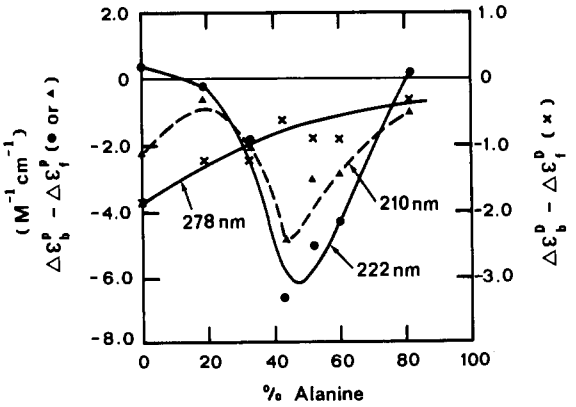

FIGURE 8: Dependence of difference CD between free and bound state for DNA (278 nm) and for protein (222 and 210 nm) upon the alanine content in the model protein.

a lack of full neutralization of phosphates in protein-bound regions by lysine residues in the proteins. The complex with poly(Lys^{48}Ala^{52}) produces an additional melting band at 110 °C in higher ionic strength, similar to that observed before for poly(Lys^{40}Ala^{60}). However, this 110 °C band was not observed for complexes with alanine content either higher or lower than 40–60%. It should be noted that model proteins containing alanine in the 40–60% range also exhibit the greatest CD changes when bound (Figures 6–8).

Figure 10 shows the CD spectra of poly(Lys^{m}Ala^n)·DNA complexes at various ionic strengths.

For poly(Lys^{19}Ala^{81})·DNA complex (Figure 10a), raising NaCl to 0.2 M results in a slight decrease in positive CD band near 275 nm. A further increase of NaCl in the medium results in a slight blue shift and enhancement of the positive CD band near 275 nm together with the appearance of a new negative band at 295 nm. The usual negative CD band at 222 nm is slightly reduced and the shoulder at 210 nm disappears altogether. Above 250 nm the effect of ionic strength on the DNA CD is qualitatively similar to that reported earlier with poly(Lys^{40}Ala^{60}), producing a transition toward A conformation (Pinkston and Li, 1974), but quantitatively the CD effect is very much smaller in poly(Lys^{19}Ala^{81}) than in poly(Lys^{40}-Ala^{60}).

Figure 10b shows the results of poly(Lys^{48}Ala^{52})·DNA complex. Here the induced changes in DNA CD above 250 nm are much greater than with poly(Lys^{19}Ala^{81}) with a more pronounced CD pattern of positive peak at 260 nm and negative peak at 295 nm resembling that of the poly(Lys^{40}Ala^{60})·DNA complex in salt (Pinkston and Li, 1974), A form DNA in ethanol (Brahms and Mommaerts 1964; Ivanov et al., 1973; Girod et al., 1973) and in DNA film (Maestre, 1970), calculated DNA CD in A conformation (Johnson and Tinoco, 1969), or A-form double-stranded RNA (Samejima et al., 1968; Wells and Yang, 1974).

For poly(Lys^{81}Ala^{19})·DNA complex (Figure 10c), where the alanine content is small, the salt-induced CD changes are completely different. The positive DNA CD band above 250 nm first red shifts with a reduction in amplitude; with increasing NaCl this reduction progressively develops into a negative CD band near 270 nm. In addition, the original 245-nm negative peak is lost and a deeper one develops near 230 nm. Increase in salt concentration also induces a gradual red shift in the positive CD of the polylysine·DNA complex and development of two big negative CD bands, one near 260 nm and another near 215 nm (Figure 10d). The salt-induced CD spectra in the latter two cases resemble those involved in the

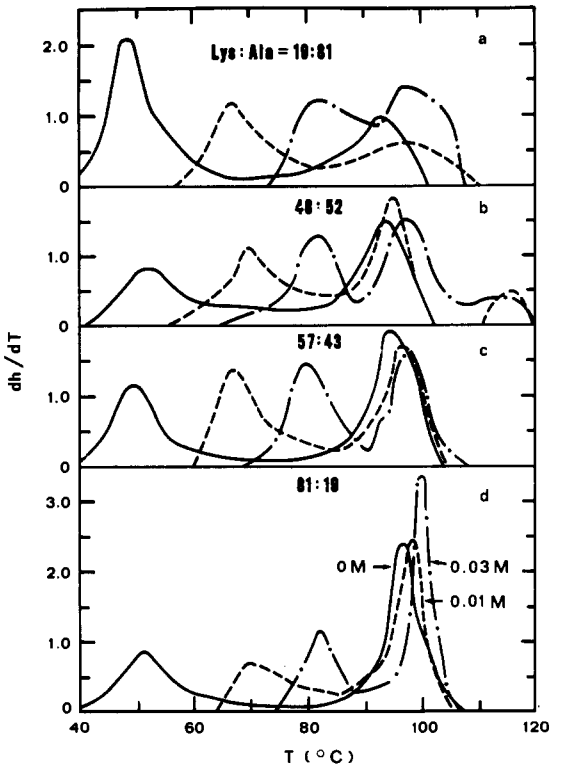

FIGURE 9: Derivative melting profiles of poly(Lys^{m}Ala^n)·DNA complexes at various ionic strengths. Both the ratio of m to n and the NaCl concentration in the medium are indicated.

B → Ψ transition reported before for polylysine·DNA in 1.0 M NaCl (Shapiro et al., 1969), for reconstituted polylysine·DNA complexes (Carroll, 1972; Li et al., 1974), reconstituted histone H1·DNA complexes in 0.14 M NaF or 0.15 M NaCl (Fasman et al., 1970; Sponar and Fric, 1972), for DNA in poly(ethylene oxide) plus NaCl (Jordan et al., 1972), and for lithium films of DNA and poly(dAT) at 92% or lower relative humidity (Brunner and Maestre, 1974).

Figure 11 shows the CD spectra of poly(Lys^{m}Ala^n)·DNA complexes as a function of r value, when complexes were made in EDTA buffer and then dialyzed to 0.2 M NaCl. The positive CD near 275 nm of poly(Lys^{19}Ala^{81})·DNA complexes (Figure 11a) is not noticeably changed when r is first increased from 0 to 0.8; with further increase it then is reduced significantly in amplitude but only slightly red shifted. This CD change is similar to that of the B → C transition observed for DNA in film (Tunis-Schneider and Maestre, 1970), in salts (Tunis-Schneider and Maestre, 1970; Li et al., 1971; Johnson et al., 1972; Ivanov et al., 1973), and in ethylene glycol (Nelson and Johnson, 1970); it is also similar to that of directly mixed polylysine·DNA (Chang et al., 1973), protamine·DNA (Yu and Li, 1973), and polyarginine·DNA complexes in EDTA (Yu et al., 1974). The protein CD near 220 nm is proportional to its r value, except at r = 3.6 where the complex is close to a full charge neutralization and resultant precipitation (Table I). The results in Figures 10a and 11a show that the slight CD change in poly(Lys^{19}Ala^{81})·DNA complex, whether toward A- or C-type spectrum, depends upon both the ionic strength and the r value of its complex.

CD spectra resembling the distorted B → A transition reported before for poly(Lys^{40}Ala^{60})·DNA complex in 0.2 M NaCl (Pinkston and Li, 1974) are also observed in poly(Lys^{48}Ala^{52})·DNA and poly(Lys^{57}Ala^{43})·DNA complexes (Figures 11b and c), all of which are in the 40–60% alanine

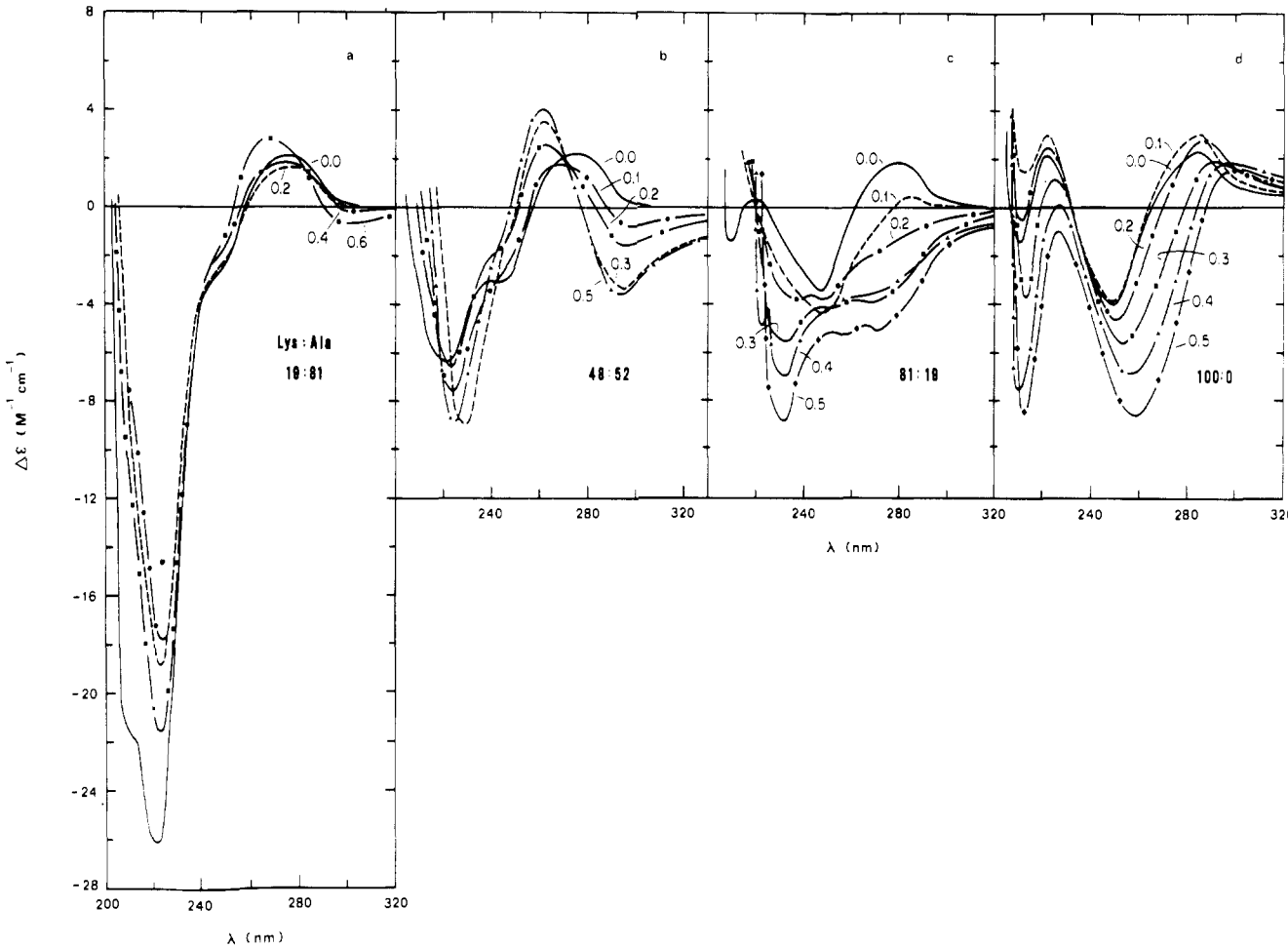

FIGURE 10: CD spectra of poly(Lys^{m}Ala^n)·DNA complexes at various ionic strengths. Both the ratio of m to n and the NaCl concentration in the medium are indicated. r is 2.7 in (a), 0.72 in (b), 0.68 in (c), and 0.6 in (d).

range mentioned earlier. If the CD change at 260 nm or at 295 nm is plotted against r, the same nonlinear dependence is obtained as reported earlier for poly(Lys^{40}Ala^{60})·DNA complexes; this could likewise be explained as a cooperative structural alteration of DNA caused by protein binding or by intermolecular interaction among the complexes.

With very little alanine content, as in case of poly(Lys^{81}Ala^{19})·DNA complexes in 0.2 M NaCl, the positive CD spectrum near 275 nm is red shifted and reduced as the r value increases, a phenomenon similar to a B → C transition. If the CD spectra in Figure 11d and in Figure 10c are compared, it becomes apparent that the B → C transition in Figure 11d may well be an intermediate of the B → Ψ transition, a suggestion made before following the investigation of difference CD spectra for B → C and B → Ψ transitions (Li et al., 1974). Figure 11d further shows that the induced CD change in this type of transition depends upon r of an order higher than one, again indicating either cooperative structural alteration of DNA by protein binding or an intermolecular interaction among the complex molecules (Pinkston and Li, 1974).

The long tails in the CD above 320 nm in Figures 10 and 11 could be attributable to light scattering in the complex solution at intermediate ionic strength, as there is a significant increase in the ratio of absorbance at 320 nm to that at 260 nm. However, although such a contribution might affect the amplitude of the CD at lower wavelengths, it is not expected that the characteristic shape of the CD spectra in Figures 10 and 11 would be changed appreciably. Therefore, interpretations of these spectra are not invalidated by the presence or absence of light scattering.

Discussion

Thermal Stability of Poly(Lys^{m}Ala^n)·DNA Complexes. One of the major concerns in the study of model protein·DNA complexes is to determine whether or not any particular nonbasic amino acid residues can potentially destabilize the DNA helix. One approach has been to examine the melting temperature of protein-bound DNA (T_m') using as a reference the T_m' of polylysine-bound DNA. It was observed that neither aromatic tyrosine residues in poly(Lys^{50}Tyr^{50}) (Santella and Li, 1974) nor alanine residues in poly(Lys^{40}Ala^{60}) (Pinkston and Li, 1974) tend to destabilize the DNA helix. On the other hand, the presence of aromatic phenylalanine in poly(Lys^{58}Phe^{42}) does destabilize the DNA helix in the course of thermal denaturation (Santella and Li, 1975). A gradual decrease in T_m' as the alanine content in the model protein is increased (Figures 1 and 3) could either be an indication of destabilization of the DNA helix by hydrophobic alanine residues, or be due to a lack of full charge neutralization on the phosphate lattice resulting from the presence of rigid α-helical structures in the model proteins having a higher alanine content. As discussed more extensively earlier (Pinkston and Li, 1974), the gradual shift of T_m' to higher temperatures in the presence of a small amount of NaCl (Figure 9) favors the second possibility. In other words, the hydrophobicity of ala-

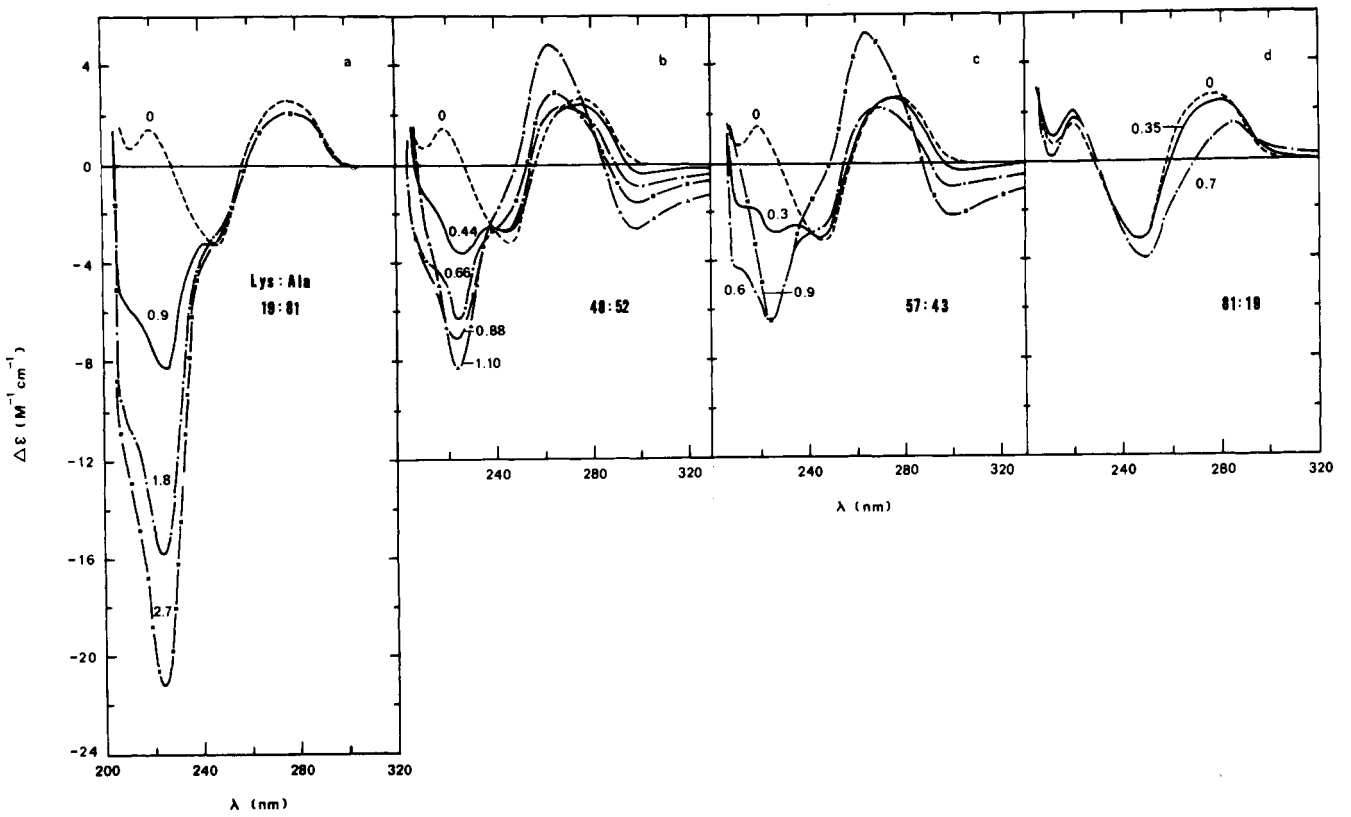

FIGURE 11: CD spectra of poly(Lys^{m}Ala^n)·DNA complexes at 0.2 M NaCl. Both the ratio of m to n and the r value of each complex are indicated.

nine residues does not in itself destabilize the DNA helix to a significant degree.

CD of Poly(Lys^{m}Ala^n)·DNA Complexes. Two main aspects of the CD results will be discussed here: the effects of complex formation on the CD of both DNA and protein, and the effect on the CD of these complexes induced by increasing the ionic strength of the medium.

The effect on CD resulting from complex formation can be divided into two parts, namely, the effect on DNA CD and the effect on protein CD.

As far as the DNA CD is concerned, at low ionic strength the directly mixed protein·DNA complexes always show some degree of red shift and a reduction of the positive CD band near 275 nm, a phenomenon characterized as a distorted B or a B → C transition (Johnson and Tinoco, 1969; Tunis-Schneider and Maestre, 1970). The extent of this transition depends upon the model protein used. The B → C transition in DNA has been considered to be a result of dehydration, because this transition, as measured by CD, can be generated by lowering humidity (Tunis-Schneider and Maestre, 1970; Li et al., 1971; Ivanov et al., 1973; Hanlon et al., 1975), or by the binding of such model proteins as polylysine (Chang et al., 1973) and other polypeptides, copolypeptides, and histones. Dehydration of DNA can occur either on the phosphate lattice or in the major or minor groove if the water molecules, bound or in the vicinity of DNA, are replaced by salts or by protein molecules. If dehydration is the main cause for this B → C transition observed in CD, the results (Figures 5 and 7) suggest that less dehydration of DNA has been effected by the binding of model proteins having higher alanine content than apparently occurs as a result of binding by polylysine. This seems surprising since the model proteins with more alanine possess more α-helical structure (Figure 6) and, therefore, are expected to be more hydrophobic. However, it is possible that the rigid α-helical structure of these model proteins cannot fit so tightly into the grooves of DNA as does polylysine, with the result that water molecules are not replaced by α-helical proteins sufficiently to effect dehydration. It is also possible that, due to this same α-helical rigidity, the phosphate residues in protein-bound regions are not fully neutralized and therefore can attract more water molecules in the vicinity of DNA. If either suggestion is correct, the results reported here seem quite compatible with the concept of a relationship between dehydration of DNA and a B → C transition in the CD spectrum.

Above 210 nm, the major CD characteristics of the α-helix do not seem to change appreciably when a model protein in this conformation is complexed with DNA, although some minor distortion does occur (Figure 6a–c). The change in amplitude of major and minor peaks in these spectra as the protein moves from a free to a complexed state can be regarded as due to an increase in α-helical content after complex formation. Below 210 nm, as a free model protein in random coil is complexed with DNA, its characteristic CD is substantially changed, (Figure 6d and e), which is not altogether unexpected because of the obvious loss of flexibility of the polypeptide chain after it is tightly bound to DNA whether it was initially in a free or a coiled state. The main effect of binding occurs below 210 nm, in the range of π,π^* transition of the amide groups (Gratzer et al., 1961; Tinoco et al., 1962, 1963; Schellman and Oriel, 1962; Holzwarth and Doty, 1965; Woody and Tinoco, 1967). Apparently the π,π^* transition is much more sensitive to protein binding on DNA than is the n,π^* transition around 220 nm, as was observed previously in a report on histone H4·DNA complexes based upon results of both vacuum uv absorption and CD (Li et al., 1971).

Model proteins with 40–50% alanine show the greatest change in the protein CD after binding to DNA (Figure 8). In the free state, these proteins contain a mixture of α helix and random coil (Figure 6); binding to DNA forces them to form more α helix at the expense of unordered coil. It may seem

unlikely that an α-helical structure formed would be the most stable equilibrium structure, yet additional α helices might permit maximum interaction between lysine residues in the model proteins and phosphates in the DNA. This would provide a possible explanation for the structural transition of DNA from the distorted B (B → C) to the A form when complexes were transferred to a medium of intermediate ionic strength.

The results in Figures 10 and 11 show that neither the alanine residue itself nor the α helix is the principal source of this kind of transition in the presence of NaCl, because poly(Lys^{19}Ala^{81}), containing the maximum percentage of both alanine and α helix of all the model proteins studied thus far, induces only a slightly detectable transition to A-type CD at high salt (Figure 10a), whereas model proteins of intermediate alanine content show a very pronounced transition to A-type CD under the same conditions (Figures 10b, 11b, and c). Possibly the additional α-helical structures forcefully formed in the process of binding (Figures 6 and 8) might have some effect on the transition of DNA structure as mentioned above.

Another possible explanation for the failure of poly(Lys^{19}Ala^{81}) to produce significant A-type CD, despite its full α helix in the free state, is that it may bind DNA in one particular groove (the major groove, for example), while the other model proteins, with less α helix in the free state, may bind DNA in another groove (such as in minor groove). This suggestion is based upon the finding that poly(Lys^{19}Ala^{81}) complexed with DNA is well protected from trypsin digestion while other model proteins, poly(Lys^{40}Ala^{60}), poly(Lys^{67}-Ala^{33}), and polylysine, complexed with DNA are not. It was therefore suggested that perhaps poly(Lys^{19}Ala^{81}) binds DNA in the major groove, and that the amide groups next to lysine residues are buried in this groove, protected from approach by trypsin, while such protection is not available for other model proteins binding DNA in the minor groove (Li et al., 1976).

In summary, this report provides the following main conclusions: (a) the binding of poly(Lys^{m}Ala^n) can induce a structural transition in DNA from B to C conformation; this structural effect diminishes as the alanine content of the model protein is increased; (b) additional α-helical structure beyond that present in the free state is induced when model proteins with 40–60% alanine are bound to DNA; (c) of all the model proteins complexed with DNA, those having 40–60% alanine undergo the largest transition to A conformation when they are transferred from low to intermediate ionic strength; (d) the complexes formed with poly(Lys^{19}Ala^{81}) undergo either a slight transition to A at higher salt or simply retain the distorted B or B → C transition at an intermediate salt, while the complexes formed with model proteins poly(Lys^{81}Ala^{19}) and polylysine, both of which have unordered structures, undergo a transition toward Ψ structure.

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Synthesis and Properties of Diastereoisomers of Adenosine 5′-(*O*-1-Thiotriphosphate) and Adenosine 5′-(*O*-2-Thiotriphosphate)†

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ABSTRACT: The chemical synthesis of adenosine 5′-(*O*-1-thiotriphosphate) (ATPαS) and adenosine 5′-(*O*-2-thiotriphosphate) (ATPβS) is described. Both exist as a pair of diastereomers, A and B. The isomers of ATPαS can be distinguished on the basis of their different reaction rates with myokinase as well as nucleoside diphosphate kinase. With both enzymes, isomer A reacts fast whereas isomer B reacts considerably more slowly. Phosphorylation of a mixture of isomers of ADPαS with pyruvate or acetate kinase yields ATPαS, isomer A, whereas the phosphoryl transfer with creatine or arginine kinase yields isomer B. The isomers of ATPβS differ in their reactivity with myosin. Isomer A is readily hydrolyzed, whereas isomer B is not. However, isomer B reacts faster with nucleoside diphosphate kinase and ADP than isomer A. Phosphoryl transfer with pyruvate kinase onto ADPβS yields ATPβS; isomer A, with acetate kinase, isomer B.

It has been shown earlier that nucleoside 2′,3′-*O*,*O*-cyclothiophosphates exist in the form of two diastereomers which in favorable cases can be separated by crystallization (Saenger and Eckstein, 1970). The investigation of these diastereomers with ribonucleases has yielded valuable information on the mechanism of pancreatic ribonuclease A (Saenger et al., 1974) as well as ribonuclease T_1 (Eckstein et al., 1972). Some time ago we have reported the synthesis of nucleoside 5′-(*O*-1-thiotriphosphates) (Eckstein and Gindl, 1970) and their polymerization by DNA-dependent RNA polymerase. It was mentioned that such nucleoside 5′-(*O*-1-thiotriphosphates) should also exist in the form of two diastereomers which at that time could not be separated. We report in this publication the enzymatic synthesis of the two single diastereomers of adenosine 5′-(*O*-1-thiotriphosphate) as well as those of a new ATP analogue, adenosine 5′-(*O*-2-thiotriphosphate). The interactions of these diastereoisomers with some ATP- or ADP-requiring enzymes will be discussed.

Experimental Section

Materials and Methods

$^{35}SPCl_3$ was purchased from Radiochemical Center, Amersham (England); [^{14}C]ADP and [^{14}C]ATP were from New England Nuclear Corp. Alkaline phosphatase (calf intestine, 5 mg/ml, 350 U/mg), snake venom phosphodiesterase (10 mg/ml, 1.5 U/mg), acetate kinase (5 mg/ml, 170 U/mg), pyruvate kinase (rabbit muscle, 10 mg/ml, 200 U/mg), nucleoside diphosphate kinase (beef liver, 5 mg/ml, 80 U/mg), myokinase (rabbit muscle, 5 mg/ml, 360 U/mg), creatin kinase (rabbit muscle, 25 U/mg), lactate dehydrogenase (rabbit muscle, 5 mg/ml, 550 U/mg), and acetate kinase (*Escherichia coli,* 1 mg/ml, 170 U/mg) were purchased from Boehringer, Mannheim (Germany). Arginine phosphate was a product of Calbiochem. Myosin (rabbit muscle) and its subfragment 1 were kind gifts of Dr. H. J. Mannherz, Heidelberg. Arginine kinase (American lobster, 2 mg/ml, 190 U/mg) was a kind gift of Dr. M. Cohn, Philadelphia. Aquasol was purchased from New England Nuclear Corp.

^{31}P nuclear magnetic resonance spectra were recorded with a Bruker Physic HFX 60 spectrometer equipped with a Fourier transform unit Bruker-Data System B-NC 12 at approximately pH 10 with proton broad-band decoupling with dilute H_3PO_4 as external standard.

Optical densities were measured with a Zeiss PMQ II. For kinetic experiments it was equipped with a Servogor recorder.

Electrophoresis was performed on paper Schleicher & Schüll 2043b (washed) in 0.1 M triethylammonium bicarbonate (pH 7.5) or 0.05 M ammonium formate (pH 3.5) with 40 V/cm for 90 min. Thin-layer chromatography (TLC) was carried out on PEI-cellulose sheets (Polygram Cel-300 polyethylenimine, uv for TLC) (Macherey and Nagel, Düren, Germany) in 0.75 M KH_2PO_4 adjusted to pH 3.5 with concentrated HCl.

When radioactive compounds were used, the amount of radioactivity in a certain product was determined by cutting

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