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Studies on Interaction between Poly(L-lysine^{58}, L-phenylalanine^{42}) and Deoxyribonucleic Acids†

Regina M. Santella and Hsueh Jei Li*

ABSTRACT: A random copolymer of 58% L-lysine and 42% L-phenylalanine, poly(Lys^{58}Phe^{42}), was used as a model protein for studying the role of phenylalanine residues in protein–DNA interaction. Complexes between this copolypeptide and DNA, made by direct mixing, were studied by absorbance, circular dichroism (CD), fluorescence, and thermal denaturation. Complex formation results in an increase in absorbance, and an enhancement, red-shift, and broadening of phenylalanine fluorescence. The fluorescence enhancement is opposite to the quenching observed when a tyrosine copolypeptide is bound to DNA (R. M. Santella and H. J. Li (1974), *Biopolymers 13,* 1909). The positive CD band of DNA near 275 nm is reduced and red-shifted by the binding of the phenylalanine copolypeptide to a greater extent than by the tyrosine copolypeptide. Thermal denaturation of the complexes in 2.5×10^{-4} *M* EDTA (pH 8.0) shows three characteristic melting bands. For complexes with calf thymus DNA, free base pairs melt at $T_{m,I}$ (47–49°) and copolypeptide-bound base pairs show two melting bands ($T_{m,II}$ at 73–75°, and $T_{m,III}$ at 88 –90°). Similar thermal denaturation results have been observed for complexes with *Micrococcus luteus* DNA. The fluorescence intensity of the complexes is greatly increased when the temperature is raised to the $T_{m,II}$ region. In addition to fluorescence measurements, the effects of increasing temperature on absorption and CD spectra of the complexes were also studied. Stacking interaction between the phenylalanine chromophore and DNA bases, either partial or full intercalation, is implicated by the experimental results. Several mechanisms are proposed to describe the reaction between the copolypeptide and DNA, and thermal denaturation of the complex.

Many biological functions directly involve interactions between DNA and proteins, such as histones and non-histone proteins (Johnson et al., 1974, Hnilica, 1972), repressors, unwinding proteins, repair enzymes, and DNA and RNA polymerases (Kornberg, 1974). At least two classes of interactions can be distinguished, nonspecific and highly specific. Perhaps the best example of nonspecific interactions are those between DNA and histones, protamine, polylysine, or polyarginine, in which the main interaction is between anionic phosphates of DNA and the cationic amino acid residues of proteins. For highly specific interactions, the most extensively studied system is between the lactose repressor and operator, in which interaction occurs between a specific protein and a specific DNA sequence (Jacob and Monod, 1961; Gilbert and Müller-Hill, 1967; Beckwith and Zipser, 1970).

During the initial stage of studies on protein–DNA interactions, both proteins and DNA were considered as two interacting macromolecules. Beyond this stage, one must look into those factors which are directly involved in the binding between these two macromolecules, such as amino acid residues and bases and the physical and chemical environments in both proteins and DNA. These questions have been dealt with from several laboratories, using polytyrosine (Friedman and Ts'o, 1971), oligopeptides (Helene et al., 1971a,b; Gabbay et al., 1972, 1973; Novak and Dohnal, 1973; Dimicoli and Helene, 1974a,b; Brun et al., 1975, Durand et al., 1975), copolypeptides with given sequences (Sponar et al., 1974; G. D. Fasman, private communication), and random sequences (Santella and Li, 1974; Pinkston and Li, 1974).

Our recent studies on the interaction between DNA and poly(Lys^{40}Ala^{60}) emphasize the effects of α-helical proteins in binding to DNA (Pinkston and Li, 1974). Studies on the interaction between DNA and poly(Lys^{50}Tyr^{50}), on the other hand, emphasize the role played by the aromatic amino acid residues (Santella and Li, 1974). As an extension of the latter report, we have studied the interaction between DNA and poly(Lys^{58}Phe^{42}). For the tyrosine chromophore, in addition to the aromatic ring, there is a hydroxyl group. The former favors stacking with bases while the latter favors hydrogen bonding with hydrogen-bond acceptors on the surface of DNA. It seems that outside interaction is favored over intercalation (Santella and Li, 1974).

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For phenylalanine, there is only a hydrophobic aromatic ring without a hydrogen bond donor. A different type of binding, presumably intercalation, could be expected. Indeed, spectroscopic and thermal denaturation results of poly(Lys^{58}Phe^{42})-DNA complexes are very different from those of poly(Lys^{50}Tyr^{50})-DNA complexes. The results of this report strongly indicate stacking interaction between bases in DNA and phenylalanine residues in poly(Lys^{58}Phe^{42}).

Materials and Methods

Copolymer L-lysine-L-phenylalanine hydrobromide (1.4:1), poly(Lys^{58}Phe^{42}), with a molecular weight of 30,000, was purchased from Miles Laboratories. It was dissolved and dialyzed against 0.001 *M* Tris (pH 6.8). The molar extinction coefficient of phenylalanine in the copolypeptide, determined by weighing the copolymer, is 210 M^{-1} cm^{-1} at 257 nm which is close to 195 M^{-1} cm^{-1} for free phenylalanine (Sober, 1973).

Calf thymus DNA was purchased from Sigma Chemical Co. and purified by phenol extraction. *Micrococcus luteus* DNA was purchased from Miles Laboratories and was used directly without further purification. Poly(adenylic acid) (poly(A)) was purchased from Miles Laboratories. The sedimentation coefficient of this poly(A) is 12.35. The concentration was determined by hydrolysis of poly(A) in 1.0 *M* NaOH using ϵ_{259} 15,000 M^{-1} cm^{-1} for AMP (Sober, 1973). Denatured calf thymus DNA was prepared by leaving a DNA solution at 100° followed by a rapid cooling. The concentration of nucleotide in DNA was determined at 260 nm using ϵ 6.5 × 10^3 M^{-1} cm^{-1} for DNA from calf thymus and 7.0 × 10^3 M^{-1} cm^{-1} for DNA from *M. luteus* (Felsenfeld and Hirschman, 1965).

Poly(Lys^{58}Phe^{42})-DNA complexes were made by the method of direct mixing, namely the slow addition of appropriate amounts of copolypeptide with a concentration of 10^{-3} *M* in amino acid residues (Lys + Phe) to 5 ml of 10^{-4} *M* DNA in 0.001 *M* Tris (pH 6.8). The input ratio of copolypeptide to DNA, *r*, is reported in amino acid residues/nucleotide. The complexes were then studied using absorption, circular dichroism (CD) and fluorescence spectroscopy, and thermal denaturation. To study the temperature effect the complexes were dialyzed to 2.5 × 10^{-4} *M* EDTA (pH 8.0).

Absorption spectra of DNA, copolypeptide, and complexes were taken on a Cary 17 spectrophotometer. CD spectra, taken on a Jasco spectropolarimeter, Model J-20, are reported as $\Delta\epsilon = \epsilon_L - \epsilon_R$ in M^{-1} cm^{-1}. For DNA and the complexes, *M* represents moles of nucleotide per liter; for free copolypeptide, moles of amino acid residues per liter. Fluorescence emission spectra of free and complexed copolypeptide, using a Perkin Elmer MPF 2A spectrofluorimeter with excitation at 250 nm, were recorded at room temperature (23 ± 2°) unless otherwise indicated.

Thermal denaturation measurements were made on a Gilford spectrophotometer, Model 2400-S, at 260 nm. The hyperchromicity, *h*, is the percent increase in absorbance at 260 nm, and d*h*/d*T* is the derivative of the melting curve.

Results

Concentration Dependence of Optical Properties of Poly(Lys^{58}Phe^{42}). It was reported earlier (Santella and Li, 1974) that a significant increase in light scattering was observed when poly(Lys^{50}Tyr^{50}) was diluted in EDTA buffer, and that no such light scattering upon dilution was detectable in Tris buffer. A similar phenomenon also occurs for poly(Lys^{58}Phe^{42}). Although a linear relationship between A_{257} and the concentration of copolypeptide from 0 to 6 × 10^{-3} *M* was found in both Tris and EDTA buffers, the straight line goes through the origin in Tris buffer but not in EDTA buffer. Because of this, the complex formation by addition of copolypeptide to DNA was made in Tris buffer instead of in EDTA buffer.

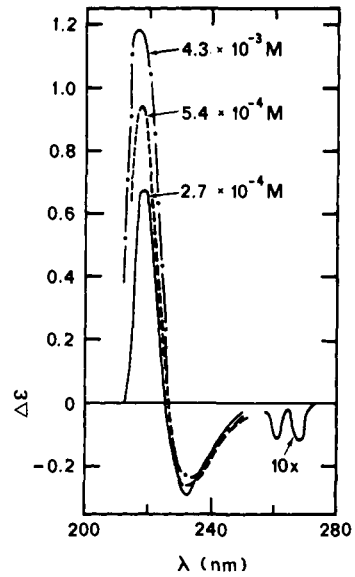

FIGURE 1: Circular dichroism spectra of poly(Lys^{58}Phe^{42}) in 0.001 *M* Tris (pH 6.8). Concentrations of copolypeptides are indicated. A 1.0-mm cell was used for solutions with concentration greater than 1 × 10^{-3} *M* while a 1.0-cm cell was used for others. For the CD near 260 nm, solutions of concentrations varied from 8.5 × 10^{-3} to 8.5 × 10^{-4} were measured either in a 1.0 or 10.0-cm cell.

Poly(Lys^{58}Phe^{42}) has a very weak CD near 260 nm with two negative peaks at 261 and 268 nm, respectively. The CD below 250 nm is significant with a negative peak at 233 nm and a positive peak at 217 nm (Figure 1). Similar results were reported previously (Peggion et al., 1972) for random-coiled copolypeptides of L-lysine and L-phenylalanine. As shown in Figure 1, the positive peak at 217 nm is sensitive to concentration while the negative peaks at 233 nm and near 260 nm are not. The concentration dependence of the CD at 217 nm could imply some intermolecular interaction between copolypeptide molecules, presumably through interaction among phenylalanine residues.

Absorption Spectra of Poly(Lys^{58}Phe^{42})-DNA Complexes. Because of the presence of lysine residues in poly(Lys^{58}Phe^{42}), the binding of this copolypeptide to DNA is expected to result in charge neutralization on the phosphate lattice of DNA and precipitation of the complexes. This has been observed for polylysine (Clark and Felsenfeld, 1971; Li et al., 1973; Itzhaki, 1974), polyarginine (Epstein et al., 1974), protamine (Yu and Li, 1973), poly(Lys^{50}Tyr^{50}) (Santella and Li, 1974), and poly(Lys^{40}Ala^{60}) (Pinkston and Li, 1974). In all these cases, precipitation occurs when the number of positively charged amino acid residues added is approximately equal to the number of negatively charged phosphates in DNA. The precipitation curve (Figure 2) shows that DNA is precipitated at r = 1.7 amino acid residues/nucleotide which is equivalent to 1.0 lysine or 0.7 phenylalanine per nucleotide. Precipitation occurs sharply at the point of full charge neutralization.

Poly(Lys^{58}Phe^{42}) has an absorption peak at 257 nm and

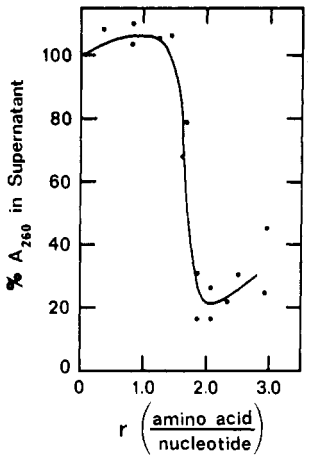

FIGURE 2: Titration curve of calf thymus DNA by poly(Lys[58]Phe[42]).

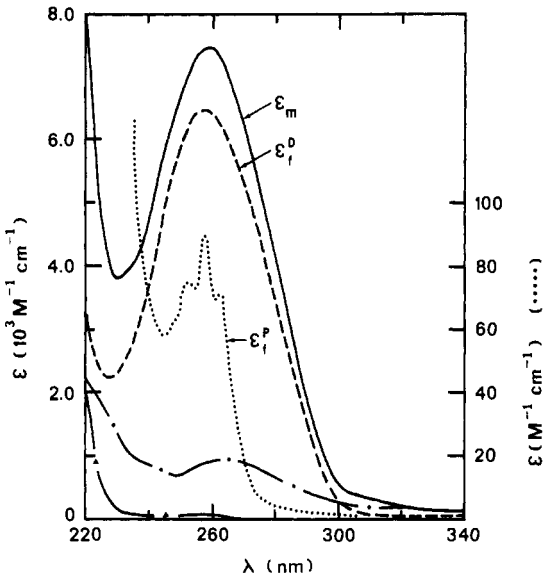

FIGURE 3: Absorption spectra of calf thymus DNA, poly(Lys[58]Phe[42]), and a complex. ϵ_f^D for DNA (- - -), and ϵ_m for the complex (r = 1.13) (—). ϵ_f^P for the free copolypeptide is given in two scales (. . . for the scale to the right and ▲ to the left). $\epsilon_m - (\epsilon_f^D + r\epsilon_f^P)$ for the absorbance gain after complex formation (●).

two subpeaks at 252 and 263 nm, respectively (Figure 3), representing the absorbance from the phenylalanine residues (Teale and Weber, 1957). The absorbance near 260 nm is extremely small compared to that of DNA. Formation of a complex between DNA and this copolypeptide results in an enhancement of absorbance. If ϵ_m, ϵ_f^D, and ϵ_f^P are, respectively, the molar extinction coefficients measured for the complex, free DNA and free copolypeptide, the gained absorbance after binding, $\epsilon_m - (\epsilon_f^D + r\epsilon_f^P)$, shows a peak at 265 nm and a greater increase below 230 nm (Figure 3). The increased absorbance near 260 nm is about ten times that of free copolypeptide and is too large to be accounted for solely by the phenylalanine chromophore. The spectral changes after complex formation, as shown in Figure 3, can be contributed by both DNA and phenylalanine.

Fluorescence Spectra of Poly(Lys[58]Phe[42])-DNA Complexes. The fluorescence maximum of free poly(Lys[58]Phe[42]) occurs at 280 nm (Figure 4a) which is identical with that of phenylalanine (Teale and Weber, 1957). Contrary to the quenching effect on tyrosine fluorescence when poly(Lys[50]Tyr[50]) is bound to DNA (Santella and Li,

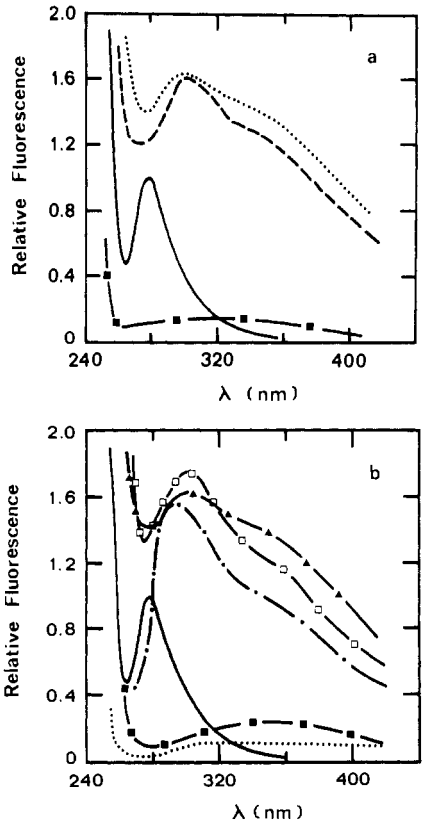

FIGURE 4: Fluorescence spectra of poly(Lys[58]Phe[42]) and its complex with nucleic acids. (a) Free copolypeptide (—) and its complex with calf thymus DNA (r = 1.35) (. . .) and with *M. luteus* DNA (- - -) (r = 1.25) and calf thymus DNA alone (■). (b) Free copolypeptide (—), with native calf thymus DNA (▲) (r = 1.35), with denatured calf thymus DNA (□) (r = 1.35), and with poly(A) (— ●—) (r = 0.75); denatured calf thymus DNA alone (■) and poly(A) alone (. . .). Excitation wavelength is 250 nm.

1974), the binding of poly(Lys[58]Phe[42]) to DNA, from either calf thymus with 42% guanine + cytosine (G + C) or *M. luteus* with 70% G + C, results in an enhancement in intensity and a red-shift and broadening of the whole spectrum. The fluorescence from DNA alone is very small compared to that of the copolypeptide or the complexes (Figure 4a).

As shown in Figure 4b, the binding of poly(Lys[58]Phe[42]) to denatured calf thymus DNA or poly(A) shows spectral changes very similar to those induced by the binding of this copolypeptide to native DNA. Again, the fluorescence of denatured DNA or poly(A) alone is very small. These results imply that the induced fluorescence spectra originate from strong electronic interaction of the phenylalanine chromophore with nucleic acids, most likely a stacking interaction with the bases.

Circular Dichroism Spectra of Poly(Lys[58]Phe[42])-DNA Complexes. Figure 5 shows the CD spectra of complexes between poly(Lys[58]Phe[42]) and DNA from calf thymus (Figure 5). The CD changes induced by copolypeptide binding show a red shift for the crossover (λ_c) near 255 nm, and a red shift and reduction of the positive band near 275 nm. Similar CD changes have been observed when *M. luteus* DNA with 70% G + C was used. These CD changes are typical of those induced by salts (Tunis-Schneider and Maestre, 1970; Li et al., 1971; Ivanov et al., 1973) and polypeptide binding (Carroll, 1972; Chang et al., 1973; Yu et al., 1974). Since the contribution from phenylalanine to

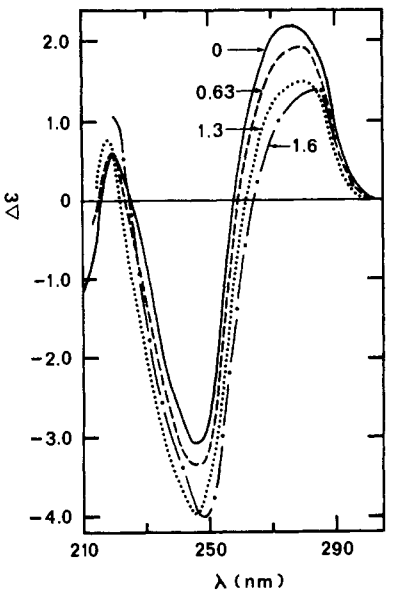

FIGURE 5: Circular dichroism spectra of poly(Lys^{58}Phe^{42})-calf thymus DNA complexes.

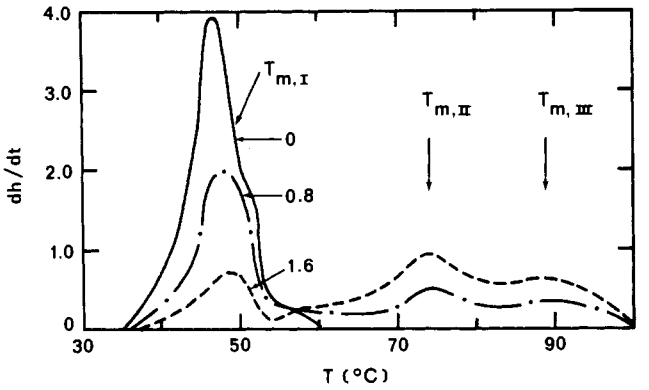

FIGURE 6: Derivative melting curves of poly(Lys^{58}Phe^{42})-calf thymus DNA complexes in 2.5 × 10^{-4} *M* EDTA (pH 8.0). *r* value for each complex is indicated. $T_{m,I}$ is assigned as the melting temperature of free base pairs, and $T_{m,II}$ and $T_{m,III}$ as those of copolypeptide-bound base pairs.

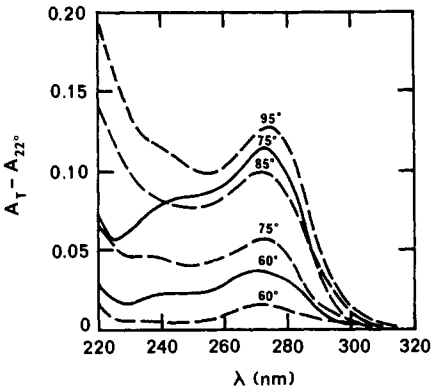

FIGURE 7: Difference absorption spectra between 22° and higher temperatures in EDTA buffer. Identical samples were placed in a reference cell at 22° and in a sample cell at elevated temperatures as indicated above each graph. Free *M. luteus* DNA (—) and a complex (- - -) with r = 1.0.

the CD above 230 nm is very small compared to that from DNA, the induced CD changes in these regions could be accounted for mainly by the DNA.

For the CD near 220 nm, although poly(Lys^{58}Phe^{42}) itself has a substantial contribution (Figure 1), the noise to signal ratios at these wavelengths are normally high, thus small changes in these regions cannot be accurately followed (Figure 5). Because of this, no attempt was made to estimate the CD of bound proteins as was done for complexes between DNA and poly(Lys^{40}Ala^{60}) (Pinkston and Li, 1974), or histone IV (Shih and Fasman, 1971; Li et al., 1971).

The CD of the complex measured either at 278 or 235 nm depends linearly upon the input ratio, *r*, of copolypeptide to DNA (Figure 5). This implies that the measured CD is approximately the sum of the CD contributed by copolypeptide-free and -bound regions. Previously, we have used thermal denaturation results of protein-DNA complexes to measure the fraction of bound base pairs and then calculated the CD of those bound base pairs. As to be shown next, since thermal denaturation properties in poly(Lys^{58}Phe^{42})-DNA complexes are more complicated than in other nucleoproteins, the analysis of the CD will be presented only after the studies on thermal denaturation are presented and discussed.

Thermal Denaturation Studies of Poly(Lys^{58}Phe^{42})-DNA Complexes. For thermal denaturation studies, the complexes were dialyzed from Tris buffer to 2.5 × 10^{-4} *M* EDTA (pH 8.0) (EDTA buffer). In EDTA buffer, in addition to $T_{m,I}$ at 47° for free base pairs, the binding of poly(Lys^{58}Phe^{42}) to DNA induces two characteristic melting bands, $T_{m,II}$ at 74° and $T_{m,III}$ at 90°. The area under the two higher melting bands is proportional to *r*, the input ratio of copolypeptide to DNA (Figure 6).

The two melting bands at $T_{m,II}$ and $T_{m,III}$ in poly(Lys^{58}Phe^{42})-DNA complexes are unique for phenylalanine binding to DNA because only one melting band at T_m' has been observed in the same EDTA buffer for DNA base pairs bound either by polylysine or by poly(Lys^{50}Tyr^{50}). In the case of poly(Lys^{50}Tyr^{50}), the complexes were also made in Tris buffer and then dialyzed to EDTA buffer before melting, as was done for poly(Lys^{58}Phe^{42})-DNA complexes in Figure 6.

If *M. luteus* DNA (70% G + C) is used instead of calf thymus DNA (42% G + C), similar melting results are obtained, except that each melting temperature is increased slightly, $T_{m,I}$ at 59°, $T_{m,II}$ at 79°, and $T_{m,III}$ at 96°, due to the higher G + C content. The ratio of the area under melting band III to that under melting band II is also independent of the G + C content. These results exclude the possibility that the two induced melting bands at higher temperature result from the melting of bound A·T and G·C pairs. After complete denaturation, the maximum hyperchromicity, h_{max}, for a complex decreases slightly when the *r* value is increased.

Temperature Effects on the Optical Properties of Poly(Lys^{58}Phe^{42})-DNA Complexes. Figure 7 shows the change in absorbance for *M. luteus* DNA and a poly(Lys^{58}Phe^{42})-DNA complex (r = 1.04) in EDTA buffer as a function of temperature. For pure DNA, the difference absorption spectra after partial (60°) and full denaturation (75°) are similar to each other with a peak at 272 ± 2 nm and a trough at 225–230 nm. The amplitude near 225 nm is about one-half of that at 270 nm. For the complex, however, the shape of the difference absorption spectrum depends upon temperature. After 75°, the amplitude at 225 nm becomes greater than the amplitude at 270 nm. Similar temperature

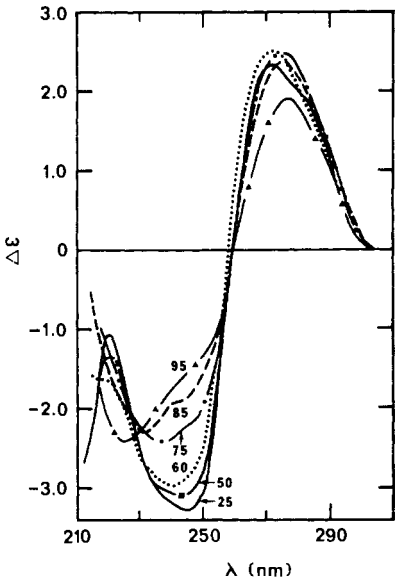

FIGURE 8: Circular dichroism spectra of poly(Lys[58]Phe[42])-*M. luteus* DNA complex (r = 0.95) at various temperatures in EDTA buffer. The temperature of each spectrum is indicated.

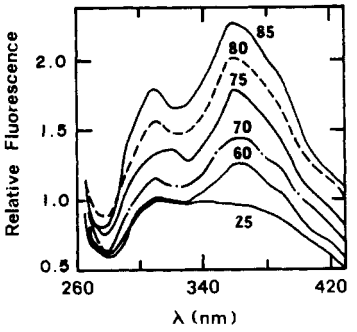

FIGURE 9: Temperature dependence of fluorescence spectra of poly(Lys[58]Phe[42])-*M. luteus* DNA complex (r = 1.04) in EDTA buffer. The temperature of each spectrum is indicated. Excitation wavelength is 250 nm.

dependence of the difference absorption spectra has also been observed for poly(Lys[58]Phe[42])-calf thymus DNA complexes.

Figure 8 shows the CD spectra of poly(Lys[58]Phe[42])-*M. luteus* DNA complexes at various temperatures. Significant CD changes occur in both the positive and the negative bands. CD spectra for *M. luteus* DNA at various temperatures were also taken. $\Delta\epsilon_{278}$ for the positive band and $\Delta\epsilon_{245}$ for the negative band for both DNA and the complex were followed in order to compare the temperature effects on CD. For pure DNA, there is a decrease of $\Delta\epsilon_{278}$ and an increase of $\Delta\epsilon_{245}$ when the DNA is denatured at 60°. CD changes accompaning premelting in DNA (Gennis and Cantor, 1972; Studdert et al., 1972) are small and were not examined in this report. For the complex, major spectral changes occur in the $T_{m,II}$ melting region (after 60°). Although there are large spectral differences between pure DNA and the complex at room temperature (Figure 5), these differences are greatly reduced at 95° when pure DNA is fully denatured and the complex is close to complete denaturation.

It was shown in Figure 4 that the binding of poly(Lys[58]Phe[42]) to DNA at room temperature results in an enhancement, red-shift, and broadening of the phenylalanine fluorescence. Figure 9 shows that, as the temperature is

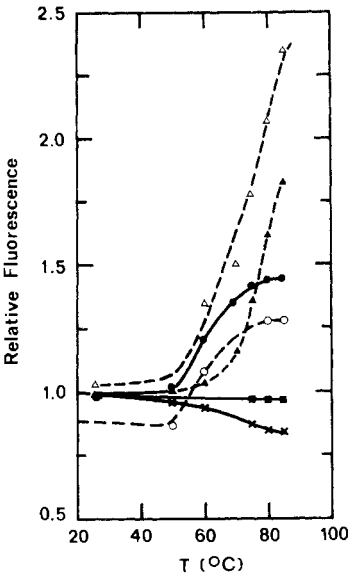

FIGURE 10: Temperature dependence of fluorescence intensity of poly(Lys[58]Phe[42])-nucleic acids complexes in EDTA buffer. Excitation wavelength is 250 nm. A complex with calf thymus DNA (r = 1.0) at 300 nm (— ● —) and at 360 nm (— ○ —); with *M. luteus* DNA (r = 1.04) at 300 nm (- - ▲ - -) and at 360 nm (- - △ - -); and at 360 nm with denatured calf thymus DNA (r = 1.35) at 300 nm (— ■ —); with poly(A) (r = 0.75) at 300 nm (— x —).

raised the broad fluorescence band (Figure 4) develops into two fluorescence peaks at 310 and 360 nm, respectively. A plot of the relative fluorescence intensity as a function of temperature (Figure 10), measured either at 300 or at 360 nm, shows a sharp increase at 75° for the complex with *M. luteus* DNA and 65° for the complex with calf thymus DNA, values in the temperature range of melting band II. For the calf thymus complex, the fluorescence intensity levels off at 80° (Figure 10), the temperature at which melting band II is completed (Figure 6). For the *M. luteus* DNA complex, the leveling off of fluorescence intensity is not apparent in Figure 10 since the highest temperature is 85°, still in the range of melting band II of this complex.

Although the binding of poly(Lys[58]Phe[42]) to denatured DNA or poly(A) results in fluorescence enhancement, red-shift, and broadening (Figure 4b) similar to that observed for native DNA, the complex of this copolypeptide with denatured DNA shows no further fluorescence enchancement up to 85°. The complex with poly(A) even shows a slight reduction in its intensity at higher temperatures (Figure 10). Parallel to these findings in fluorescence, no substantial hyperchromicity in absorbance at 260 nm has been observed from 25 to 100° for complexes of poly(Lys[58]Phe[42]) with denatured DNA (unpulished results).

Analysis of Thermal Denaturation and Circular Dichroism Results. $A_{T_{m,I}}$ and A_T respectively are defined as the area under melting band I and the total area under the whole melting curve. If it is assumed that the fraction of melting area at $T_{m,I}$, namely $A_{T_{m,I}}/A_T$, is approximately proportional to the fraction of free base pairs in the complex, the following equation can be used:

$$1 - F = \frac{A_{T_{m,I}}}{A_T} \quad (1)$$

where F is the fraction of base pairs bound by the copolypeptide.

After F is determined from eq 1 using melting results, β,

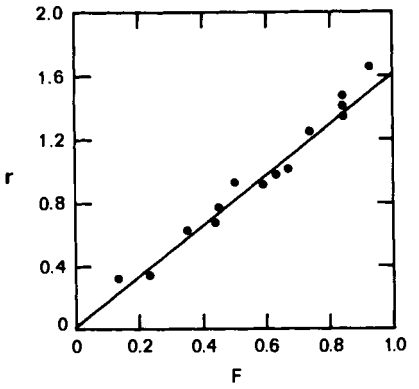

FIGURE 11: Linear plots of eq 2.

the ratio of amino acid residues per nucleotide in the copolypeptide-bound regions, can be determined from the plot of the following equation (Li, 1973):

$$r = \beta F \quad (2)$$

The plot of eq 2 is shown in Figure 11 and the slope (β) is determined to be 1.6 amino acid residues per nucleotide, equivalent to 0.93 lysine and 0.67 phenylalanine residue per nucleotide. For poly(Lys^{50}Tyr^{50}), the β value is 2.0 amino acid residues per nucleotide, equivalent to 1.0 lysine and 1.0 tyrosine residue per nucleotide (Santella and Li, 1974).

The consistent reduction of the DNA CD in copolypeptide–DNA complexes with higher r values (Figure 5) indicates that it can be decomposed into two components, $\Delta\epsilon_f^D$ of free base pairs and $\Delta\epsilon_b^D$ of copolypeptide-bound base pairs. In other words, the measured CD, $\Delta\epsilon_m$, of each complex is a sum of these two components:

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

$\Delta\epsilon_b^D$ is then calculated for complexes with varied r values. The results for two complexes are shown in Figure 12 with a consistent $\Delta\epsilon_b^D$, having a positive maximum at 280 nm (λ_{max}), a crossover at 263 (λ_c) and $\Delta\epsilon_{280} = 1.5$, about two-thirds of that of pure DNA at the same wavelength. As far as the CD of bound DNA is concerned, the CD changes in DNA due to binding follows the order of polylysine > poly(Lys^{58}Phe^{42}) > poly(Lys^{50}Tyr^{50}) (Chang et al., 1973; Santella and Li, 1974).

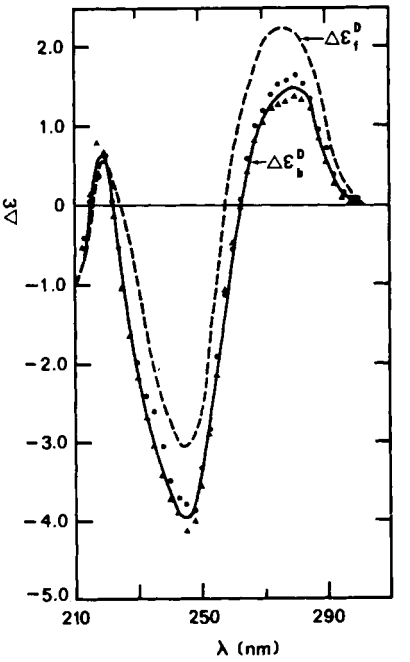

FIGURE 12: Calculated CD spectrum, $\Delta\epsilon_b^D$, for calf thymus DNA bound by poly(Lys^{58}Phe^{42}). $\Delta\epsilon_b^D$ was calculated from eq 3, using a complex with $r = 0.66$ (●) and $r = 1.32$ (▲). The buffer is 0.001 M Tris (pH 6.8). Also included is $\Delta\epsilon_f^D$ of free calf thymus DNA.

Discussion

Interaction between the Phenylalanine Chromophore and DNA. Before the mechanisms of interaction between the phenylalanine chromophore and DNA are discussed, the pertinent results and their implications can be summarized as follows.

(1) When poly(Lys^{58}Phe^{42}) is complexed with native DNA (Figure 3), there are substantial changes in absorbance both in the 260-nm region and below 230 nm, indicating a strong electronic interaction between the phenylalanine chromophore and DNA.

(2) Opposite to fluorescence quenching as expected from both reabsorption of phenylalanine fluorescence by bases and energy transfer from phenylalanine to bases, phenylalanine fluorescence is greatly enhanced when the copolypeptide is bound to native or denatured DNA and to single-stranded poly(A) (Figure 4a and b). In addition, the fluorescence spectrum of phenylalanine is red-shifted and broadened after binding. These spectral changes suggest that the complexed phenylalanine has a new electronic configuration different from the original chromophore. These results imply a strong electronic interaction of phenylalanine chromophores with nucleic acids, most likely a stacking interaction with bases. This interaction has occurred in many systems when both interacting molecules have aromatic structures.

(3) Spectral changes in absorbance at $T_{m,II}$ for the complex with native DNA are similar to those observed when the copolypeptide is bound to the native DNA at room temperature. No such spectral changes at higher temperatures have been observed when denatured DNA was used for the initial complexes (unpublished results). These results imply that the spectral changes near the $T_{m,II}$ are related to the double helical structure of native DNA in the complex, and that further interaction between phenylalanine and native DNA occurring at $T_{m,II}$ is similar to the one which occurs when the copolypeptide is first complexed with native DNA at room temperature.

(4) Fluorescence changes at elevated temperatures occur mainly at $T_{m,II}$. These changes are similar to those observed for the complexes at room temperature, indicating further interaction at $T_{m,II}$ similar to that at room temperature when the two macromolecules are mixed together. No such fluorescence changes have been observed at higher temperatures for the complexes with denatured DNA or with poly(A) (Figures 9 and 10).

Based upon these facts and their implications, the following three mechanisms could be suggested to describe the events occurring in the phenylalanine chromophore when the copolypeptide is complexed with native DNA and when the complex is thermally denatured.

If the distribution of phenylalanine and lysine residues are approximately random along the copolypeptide, as indicated by the specifications of Miles Laboratories, one melting band corresponding to the denaturation of copolypeptide-bound regions would be expected. This has been ob-

served in many other systems such as polylysine, polyarginine, protamine, and other model proteins. Under this assumption, the following two mechanisms of eq 4 and 5 are suggested:

$$P + D \longrightarrow (PD)_{out} \xrightarrow{T_{m,II}} (PD)_{in} \xrightarrow{T_{m,III}} (PD_{den}) \quad (4)$$

where $(PD)_{out}$ is the partially intercalated complex at room temperature; $(PD)_{in}$ is the further intercalated complex at $T_{m,II}$ and (PD_{den}) is the denatured complex at $T_{m,III}$. In other words, melting band III corresponds to the ordinary denaturation of copolypeptide-bound regions, while melting band II does not.

This mechanism is supported by the experimental results summarized above. They suggest that the interaction at $T_{m,II}$, $(PD)_{in}$ (intercalation), is a further reaction of the type at room temperature, $(PD)_{out}$ (partial intercalation), when both macromolecules interact with each other by direct mixing.

Theoretically, this mechanism is not unreasonable. As discussed previously (Santella and Li, 1974), intercalation of aromatic amino acid residues in native DNA could result in overlapping between the aromatic ring with the bases on only one of the two strands of DNA, and in unstacking of the bases on both strands. Although stacking interaction between the chromophore of the aromatic amino acid residues and the bases of one of the two strands could possibly be energetically favorable, the unstacking of bases on the other strand is energetically unfavorable. Consequently full intercalation of the chromophore of aromatic amino acid residues in native DNA might not occur as readily as do the acridine dyes and actinomycins, in which the multiple rings can overlap with base pairs on both strands of DNA. The mechanism of eq 4 suggests only partial intercalation of the phenylalanine chromophore to native DNA upon binding. Further intercalation could occur at $T_{m,II}$ when the base pairs are further unstacked at higher temperatures. Finally, the complex is denatured at $T_{m,III}$ when native base pairs cannot be maintained.

A second mechanism could also be suggested:

$$P + D \longrightarrow PD \xrightarrow{T_{m,II}} PD_{den} \xrightarrow{T_{m,III}} P + D_{den} \quad (5)$$

Here a native complex, PD, with partial or full intercalation is formed at room temperature; it is denatured at $T_{m,II}$ with the phenylalanine chromophore stacked with denatured DNA; finally, the stacked complex is destroyed at $T_{m,III}$.

This mechanism is supported by some of the experimental results summarized above. However, if the mechanism of eq 5 were correct, one would expect a melting band at $T_{m,III}$ for a complex of poly($Lys^{58}Phe^{42}$) with denatured DNA. No such melting band has been observed when the absorbance change was monitored at 260 nm (unpublished results). Therefore, the mechanism of eq 5 is less likely than the mechanism of eq 4.

A third mechanism could also be proposed upon the assumption that the distribution of phenylalanine and lysine residues along the copolypeptide molecule were nonrandom: roughly one-half of the molecule could be rich in lysine (L) and the other half rich in phenylalanine (P). The binding of this copolypeptide P-L to DNA (D-D) yields two types of complexed regions, PD and LD. PD is denatured at $T_{m,II}$ and LD at $T_{m,III}$ because the binding of lysine rich regions (L) to phosphates yields a greater stabilization on DNA (eq 6). The mechanism of eq 6 is also in agreement with the experimental results. One could assign spectral changes in absorbance and fluorescence after binding at room temperature to the formation of PD with partial or full intercalation and assign the spectral changes at $T_{m,II}$ to the denaturation of PD to PD_{den}. The fact that there is no substantial change in fluorescence at $T_{m,III}$ could be explained as a result of low phenylalanine content in LD regions.

$$\begin{array}{c} P \\ | \\ L \end{array} + \begin{array}{c} D \\ | \\ D \end{array} \longrightarrow \begin{array}{c} PD \\ || \\ LD \end{array} \xrightarrow{T_{m,II}} \begin{array}{c} PD_{den} \\ || \\ LD \end{array} \xrightarrow{T_{m,III}} \begin{array}{c} PD_{den} \\ || \\ LD_{den} \end{array} \quad (6)$$

The mechanism of eq 6 could be true only if (a) the distribution of lysine or phenylalanine along the molecule is indeed very uneven and (b) the fluorescence intensity of a denatured complex is greater than the original native complex. These two conditions cannot be proved or disproved. Consequently, based upon our present results, the mechanisms of eq 4 and 6 cannot be distinguished. Nevertheless, the mechanism of eq 4 could be favored over that of eq 6, because the former is theoretically reasonable and the polymerization procedure presumably yields a random copolypeptide (from Miles Laboratories).

Comparison with Other Systems. Fluorescence quenching has been observed when tyrosine or tryptophan containing oligopeptides are complexed with DNA or poly(A) (Brun et al., 1975). A similar quenching effect has also been observed when a copolypeptide of tyrosine, poly($Lys^{50}Tyr^{50}$), was used (Santella and Li, 1974). Therefore, the observed fluorescence enhancement, red-shift, and broadening when a phenylalanine copolypeptide, poly($Lys^{58}Phe^{42}$), is complexed to native or denatured DNA or to single-stranded poly(A) is unique for phenylalanine.

Some valuable information with regard to the roles played by aromatic amino acid residues in binding to DNA has been gained from studies on the interaction between nucleic acids and oligopeptides containing aromatic amino acid residues (Gabbay et al., 1972, 1973; Helene et al., 1971a,b; Novak and Dohnal, 1973; Dimicoli and Helene, 1974a,b). Nevertheless, substantial differences could possibly occur if an aromatic containing copolypeptide is used rather than an oligopeptide. For instance, based upon nuclear magnetic resonance studies of phenylalanine containing oligopeptides, Gabbay et al. (1972, 1973) suggested partial intercalation of phenylalanine in DNA which, in part, agrees with our conclusion. Nevertheless, they reported that these oligopeptides did not induce any detectable changes in absorption and CD spectra of DNA, which are different from the results in this report. These differences could be due to a greater contribution of $-NH_3^+$ and $-COO^-$ at both ends to the binding of oligopeptides to DNA than of copolypeptides. The presence of greater amounts of lysine residues in copolypeptides could also result in very strong and localized binding of these copolypeptides to DNA, while the oligopeptides could bind DNA in a nonlocalized fashion because of their weaker binding affinity.

It is interesting to compare the CD spectra of poly($Lys^{58}Phe^{42}$)-DNA in Figures 5 and 12 and poly($Lys^{50}Tyr^{50}$)-DNA in Figure 5 of Santella and Li (1974). Although these two copolypeptides have similar lysine contents, the induced CD effects on DNA are much greater for the copolypeptide with phenylalanine than for that with tyrosine. They could result from different modes of binding of these two copolypeptides to DNA, outside binding for tyrosine and intercalation for phenylalanine. It could also be due to different dehydration effects in the grooves of DNA by these two copolypeptides.

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