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

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ABSTRACT: Thermal denaturation of DNA with varied GC contents, synthetic copoly(dAT) and poly(dG)·poly(dC) complexed with polyarginine, and calf thymus DNA complexed with poly(Arg^{87},Orn^{13}) in 2.5 × 10^{-4} M EDTA (pH 8.0) has been studied. For polyarginine–natural DNA complexes, in addition to the melting band at T_m of free base pairs, there are two melting bands at T_m', $T_{m,I}'$ and $T_{m,II}'$ of bound base pairs. The ratio of melting area at $T_{m,II}'$ to that at $T_{m,I}'$ decreases as the GC content of DNA increases. For copoly(dAT), in addition to the melting band at T_m, the binding of polyarginine induces only one melting band at T_m'. For poly(dG)·poly(dC), the presence of polyarginine yields only a monophasic melting at T_m. The presence of 13% ornithine in polyarginine, namely poly(Arg^{87},Orn^{13}), also induces two melting bands at T_m' on calf thymus DNA. Nevertheless, the ratio of melting area at $T_{m,II}'$ to that at $T_{m,I}'$ is much greater for poly(Arg^{87},Orn^{13}) than for polyarginine. The requirements of GC content and secondary structure of DNA for a specific binding by polyarginine are discussed.

In the past decade basic polypeptide–DNA interaction has been actively studied using thermal denaturation (Tsuboi *et al.*, 1966; Leng and Felsenfeld, 1966; Olins *et al.*, 1967, 1968; Kawashima *et al.*, 1969; Inoue and Ando, 1970; Shih and Bonner, 1970; Li *et al.*, 1974a,b,d) and circular dichroism (CD) (Haynes *et al.*, 1970; Carroll, 1972; Chang *et al.*, 1973; Yu and Li, 1973). Previously the main concern of thermal denaturation of the basic polypeptide–DNA complex was its biphasic melting where free DNA melts at lower temperature (T_m) while polypeptide-bound DNA melts at higher temperature (T_m'). Recently a new concept was introduced (Li, 1973) that, in a basic protein–DNA complex, base pairs with sufficiently different thermal stability from their neighbors can melt at different temperatures. With this concept new information of protein–DNA interaction has been revealed in chromatin (Li and Bonner, 1971; Li, 1973; Li *et al.*, 1973) in polylysine–DNA complex (Chang *et al.*, 1973) and in protamine–DNA complex (Yu and Li, 1973).

This report and the subsequent one (Yu *et al.*, 1974) extend our previous work using thermal denaturation and circular dichroism to study interaction between polyarginine and DNA of varied GC (guanine + cytosine) contents. Thermal denaturation results are presented in this communication and CD results in the subsequent report (Yu *et al.*, 1974).

For a direct mixed polyarginine–calf thymus DNA complex, in addition to the melting band at T_m (50 ± 2°) for free base pairs, there are two melting bands at T_m', $T_{m,I}'$ at 91 ± 1.5°, and $T_{m,II}'$ at 99 ± 2° for bound base pairs. This phenomenon has consistently been observed for complexes of polyarginine with DNA of varied GC contents. $T_{m,I}'$ and $T_{m,II}'$, and the ratio of their melting areas, however, depend upon the GC content and possibly the secondary structure of DNA. The presence of 13% ornithine in polyarginine makes the melting properties of its complex with DNA quite different. It is suggested that the presence of two melting bands at $T_{m,I}'$ and $T_{m,II}'$ is due to the binding of polyarginine to the two opposite grooves of DNA.

Materials and Methods

Calf thymus DNA was purchased from Sigma Chemical Co. and was purified by phenol extraction. *Micrococcus luteus* DNA, copoly(dAT) and poly(dG)·poly(dC) were purchased from Miles Laboratories, *Escherichia coli* (strain B) and *Clostridium perfringens* DNA from Sigma Chemical Co. These were used directly without further purification. GC content of DNA is considered to be 0% for copoly(dAT), 31% for *Cl. perfringens*, 42% for calf thymus, 53% for *E. coli*, 70% for *M. luteus*, and 100% for poly(dG)·poly(dC) (Sober, 1970). The molar extinction coefficient of DNA at 260 nm is 7,400 M^{-1} cm^{-1} for *Cl. perfringens*, 6500 for *E. coli*, 7000 for *M. luteus* (Felsenfeld and Hirschman, 1965), 6800 for copoly(dAT), 6200 for poly(dG)·poly(dC) (provided by Miles Laboratories), and 6500 for calf thymus. Stock solutions of DNA were made in EDTA buffer (2.5 × 10^{-4} M EDTA (pH 8.0)).

Poly(L-arginine) sulfate (mol wt 14,000) was purchased from Miles Laboratories. The purity was confirmed by amino acid analysis as 99% in arginine. Poly(L-arginine) hydrochloride (mol wt 65,000) was purchased from Sigma Chemical Co. Amino acid analysis showed that this sample happened to contain 13% ornithine. In this report this sample was used as poly(Arg^{87},Orn^{13}). Poly(L-ornithine) hydrobromide (mol wt 32,400) was purchased from Miles Laboratories. Stock solutions of polyarginine, poly(Arg^{87},Orn^{13}) and polyornithine were made in and dialyzed to EDTA buffer before use. Their concentrations were determined both by ninhydrin method (Spies, 1957), using arginine as the standard, and amino acid analysis, using modified automated Amino Acid Analyzer (Liao *et al.*, 1973).

The complexes between DNA and polyarginine or poly(Arg^{87},Orn^{13}) were made exactly in the same manner as in polylysine–DNA complexes by a slow and direct mixing at room temperature (Li *et al.*, 1972). For copoly(dAT), the complexes were made at 4° in cold room. After the complexes were made in EDTA buffer and centrifuged at 10,000 rpm in a Sorvall

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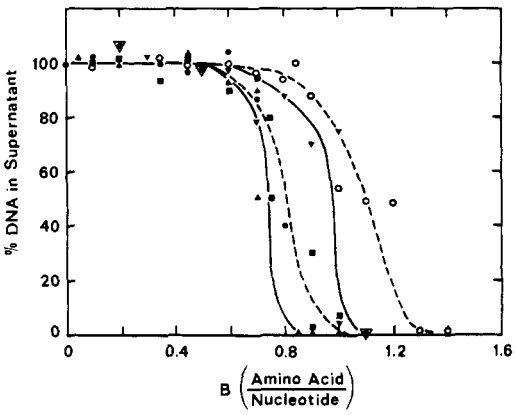

FIGURE 1: Titration curves of DNA with varied GC contents by polyarginine and poly(Arg^{87},Orn^{13}). Titrated by polyarginine is DNA from *Cl. perfringens* (O- - -O), calf thymus (■- - -■), *E. coli* (▲-▲), and *M. luteus* (●- - -●). Titrated by poly(Arg^{87}Orn^{13}) is DNA from calf thymus (▼——▼).

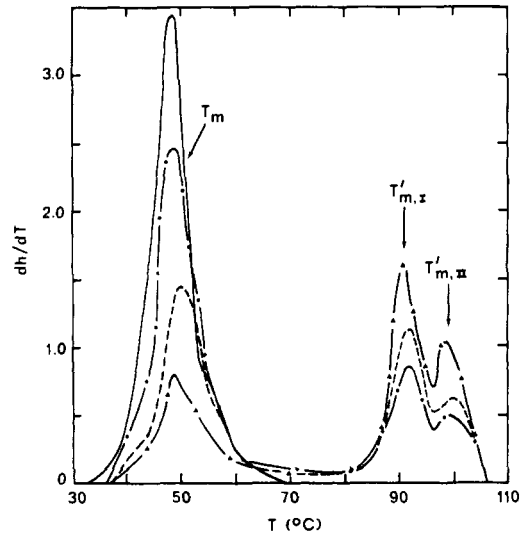

FIGURE 2: Derivative melting profiles of polyarginine–calf thymus DNA complexes. B = 0 (——), 0.30 (· —), 0.45 (- - -), and 0.60 (- ▲ -).

SS-34 rotor at 4° for 10 min, the supernatants were collected and studied. For the experiments of ionic strength effect on melting of the complex the supernatant was dialyzed to EDTA buffer with appropriate concentrations of NaCl. The input ratio, B, of polyarginine or poly(Arg^{87},Orn^{13}) to DNA in each complex is reported in amino acid residues per nucleotide.

The absorbance and thermal denaturation measurements of the supernatants were obtained using a Gilford spectrophotometer Model 2400-S. The derivative plots dh/dT of the melting curves are reported as done before (Li and Bonner, 1971). h is the per cent increase in absorbance after heating referred to the absorbance at room temperature. Hyperchromicity was measured at 260 nm except in one experiment in which 250 and 280 nm were used. In that case the wavelengths used will be indicated.

Results

Binding of Polyarginine and Poly(Arg^{87},Orn^{13}) to DNA of Varied GC Contents. At low salt DNA is bound randomly and irreversibly by polylysine. The complexes are soluble until the B value of the complex reaches 1.0 lysine/nucleotide (Clark and Felsenfeld, 1971; Li *et al.*, 1973). For polyarginine and poly(Arg^{87},Orn^{13}), the midpoint of precipitation curve depends upon the DNA and the polypeptide used (Figure 1). For polyarginine–DNA complexes, the midpoint of precipitation is 0.75 for *E. coli* DNA and 0.80 for both calf thymus and *M. luteus* DNA at which the charges on DNA phosphates are not fully neutralized. For *Cl. perfringens* DNA, on the other hand, precipitation occurs at B = 1.1 when phosphates on DNA are more than neutralized. The midpoint of the precipitation curve for poly(Arg^{87},Orn^{13})-calf thymus DNA is 0.98 which is closer to a value of 1.0–1.05 for polylysine-calf thymus DNA complexes (Clark and Felsenfeld, 1970; Li *et al.*, 1973) than 0.80 for polyarginine-calf thymus DNA complexes (Figure 1). Apparently charge neutralization is not the only factor controlling precipitation of polyarginine-DNA complex.

Thermal Denaturation of Polyarginine–Calf Thymus DNA Complexes. Typical derivative melting curves of polyarginine–calf thymus DNA are shown in Figure 2. As more polyarginine is bound to DNA, the melting band at T_m (about 50°) is decreased. This phenomenon is true for every basic polypeptide–DNA complex. Polyarginine–DNA complex differs from polylysine–DNA (Tsuboi *et al.*, 1966; Li *et al.*, 1973) or protamine–DNA complex (Yu and Li, 1973) in that there appear two melting bands at T_m' region, $T_{m,I}'$ at about 91° and $T_{m,II}'$ at about 100°, for the former (Figure 2), and only one melting band at T_m' for the latter.

The existence of two melting bands at T_m' region for polyarginine–calf thymus DNA complex is not an artifact of EDTA buffer used for the complexing and melting experiment. Two melting bands at T_m' were also observed when the complexes were made and examined at 0.005 M cacodylate buffer, pH 7.0.

In EDTA buffer, the ratio of melting areas of $T_{m,II}'$ to $T_{m,I}'$ varies from 0.40 to 0.46 (Table I) as the B value of the complex is changed. The other pertinent melting parameters of polyarginine–calf thymus DNA complexes are also included in Table I. A complex with a higher B value has a lower h_{max} which is similar to that observed in direct mixed polylysine–DNA (Li *et al.*, 1973) and protamine–DNA complexes (Yu and Li, 1973). Light scattering, as indicated by A_{320}/A_{260}, is small and is not significantly changed before and after melting.

The first question asked about the two melting bands at T_m' region is whether some polyarginine-bound regions are electrostatically more neutralized than the others, such that they end up with two different thermal stabilities. This possibility is tested by the experiment of Figure 3. A polyarginine–calf thymus DNA with B = 0.45 was made in EDTA buffer and then

TABLE I: Melting Parameters of Polyarginine–Calf Thymus DNA Complexes.

B (Amino Acid/ Nucleotide)	h_{max}[a] (%)	T_m	$T_{m,I}'$	$T_{m,II}'$	$A_{T_{m,II}'}/A_{T_{m,I}'}$
0	35.9	48.5			
0.10	30.2	48.5	90.5	100	
0.20	29.6	49.0	89.5	97	0.46
0.30	32.2	48.5	91.5	99.5	0.43
0.45	28.3	50.0	92.0	100.0	0.41
0.60	25.7	49.0	91.0	99.0	0.40
0.70	23.7	51.5	92.5	100.5	0.43

[a] h_{max} is the maximum hyperchromicity when melting is complete.

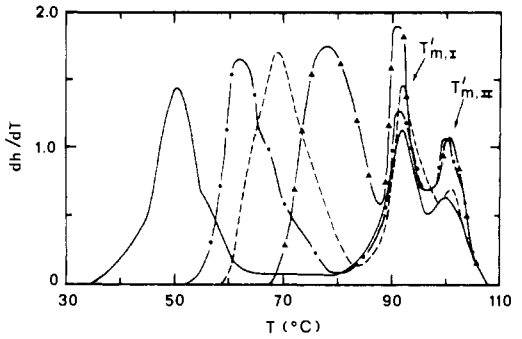

FIGURE 3: Effect of ionic strength on melting of polyarginine-calf thymus DNA complex. B = 0.45. The buffer is EDTA plus NaCl of 0 (——), 0.005 (- · -), 0.01 (- - -), and 0.05 M (- ▲ -).

dialyzed to buffers with EDTA plus 0.005, 0.01, and 0.05 M NaCl, respectively. The melting temperature of free base pairs at T_m is raised as the ionic strength of the solution is increased. On the other hand, the melting temperatures, $T_{m,I}'$ and $T_{m,II}'$, of bound base pairs are not shifted. Similar results have been found in direct mixed protamine-DNA complex (Yu and Li, 1973) and polylysine-DNA complex (Li *et al.*, 1974b). These results indicate that electrostatic shielding on those bound pairs, melting at $T_{m,I}'$ and $T_{m,II}'$, are not substantially different.

Thermal Denaturation of Complexes between Polyarginine and DNA of Varied GC Contents. Another possible explanation for two melting bands of polyarginine-bound base pairs at T_m' is that they represent two thermal stabilities of the bound regions, such as GC-rich and AT-rich regions in the complex. In order to explore this possibility thermal denaturation of complexes between polyarginine and DNA of varied GC contents was studied. Figure 4 shows some typical results of polyarginine-*M. luteus* DNA complexes. *M. luteus* DNA contains 70% GC which is the highest of the natural DNA studied in this report. As shown in Figure 4, two melting bands at T_m' region are still apparent. Compared with those two bands in polyarginine-calf thymus DNA complexes (Figure 2), the

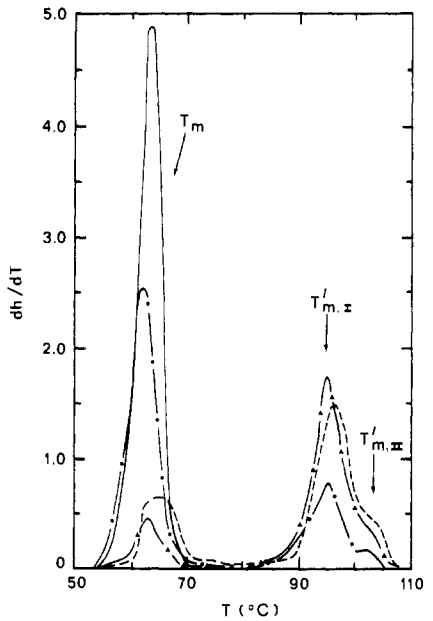

FIGURE 4: Derivative melting profiles of polyarginine-*M. luteus* DNA complexes. B = 0 (——), 0.35 (- · -), 0.60 (- - -), and 0.70 (- ▲ -).

TABLE II: Melting Parameters of Polyarginine–Bacterial DNA Complexes.

DNA	B (Amino Acid/ Nucleotide)	h_{max}[a] (%)	T_m	$T_{m,I}'$	$T_{m,II}'$	$A_{T_{m,II}'}/A_{T_{m,I}'}$
M. luteus	0	25.3	61.8			
	0.20	22.6	62.5	95.3	101.8	0.12
	0.45	21.9	63.5	96.5	105.0	0.04
	0.60	21.4	63.5	96.5	103.5	0.12
	0.70	20.1	63.0	96.5	105.5	0.05
E. coli	0	27.0	50.5			
	0.20	25.5	49.5	91.5	98.0	0.40
	0.45	21.8	50.3	90.8	98.0	0.30
	0.60	21.8	52.3	92.5	99.3	0.31
	0.70	18.7	52.0	92.3	99.3	0.31
Cl. perfringens	0	24.8	39.2			
	0.20	24.4	39.0	84.8	93.8	0.43
	0.45	22.1	39.0	86.5	96.0	0.50
	0.70	21.9	39.5	88.5	97.8	0.53
	0.90	20.3	39.8	88.5	97.3	0.56

[a] See footnote of Table I.

$T_{m,II}'$ band is greatly reduced in *M. luteus*. It indicates that the ratio of area at $T_{m,II}'$ to that at $T_{m,I}'$, depends upon the GC content of DNA used. In fact, this is shown to be true in *E. coli* DNA (53% GC) and *Cl. perfringens* DNA (31% GC). The pertinent melting results of these complexes are summarized in Table II. It is seen that, as expected, h_{max} is reduced for complexes with higher B values. T_m, $T_{m,I}'$, and $T_{m,II}'$ increase with respect to GC content of DNA used. On the other hand, the ratio of melting area of the two melting bands, $A_{T_{m,II}'}/A_{T_{m,I}'}$, decreases for DNA with higher GC.

It is clear from the above results that the two melting bands at $T_{m,I}'$ and $T_{m,II}'$ are related to the GC content of polyarginine-bound regions in the complex. Because of this, complexes between polyarginine and double-stranded synthetic copoly(dAT) and poly(dG)·poly(dC) were studied. The results of copoly(dAT) are shown in Figure 5 and Table III. For pure copoly(dAT) there is a big and very sharp melting at 23.5° and a small and broad melting at 36°. The highly cooperative melting indicates that copoly(dAT) is a double-stranded DNA. As more polyarginine is added to the copoly(dAT), the melting of free base pairs is reduced with an increase of melting of bound base pairs at T_m' (72°). No second band of bound base pairs was detected even though the temperature was raised to 110°. This observation is quite mysterious because the earlier results imply that the second melting band at $T_{m,II}'$ becomes more significant as the AT content of DNA is increased. Here it shows no $T_{m,II}'$ for polyarginine-copoly(dAT) complexes when the DNA has 100% AT. Though the results do not agree with the earlier expectation the experiment does encourage us to look into the complex formation more seriously and critically. This will be discussed later.

Other striking results are found in the complexes of polyarginine with poly(dG)·poly(dC) as shown in Figure 6. The free DNA has a sharp melting at 66.5° with h_{max} of 18.2 at 260 nm and 50.8 at 280 nm (Table III). This indicates that the DNA is double-stranded. When polyarginine was added to the DNA, the absorbances at 260 nm of the supernatants of the mixtures

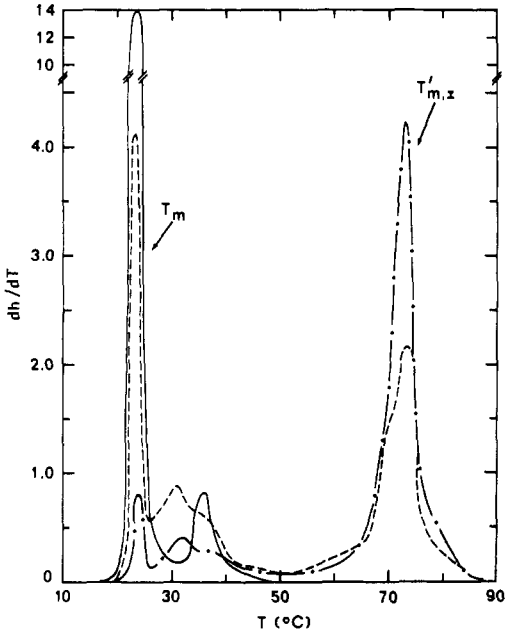

FIGURE 5: Derivative melting profiles of polyarginine-copoly(dAT) complexes. B = 0 (——), 0.90 (- - -), and 1.60 (- · -).

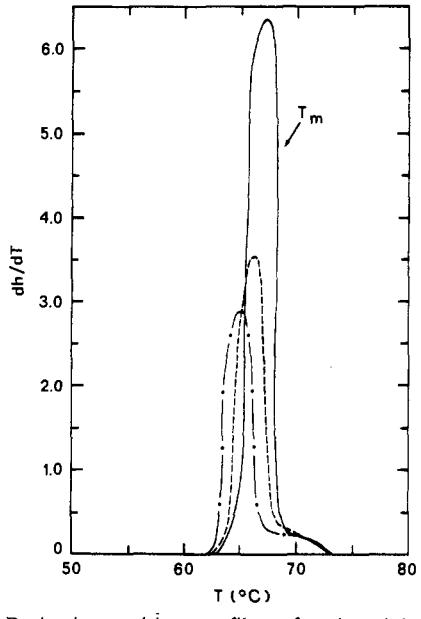

FIGURE 6: Derivative melting profiles of polyarginine-poly(dG)·poly(dC) complexes. B = 0 (——), 0.40 (- - -), and 0.60 (- ● -).

show that all poly(dG)·poly(dC) (95–100%) was still in solution. Nevertheless, there is no biphasic melting detected at 260 or 280 nm when the temperature was raised to 117°. The presence of polyarginine reduces h_{max} (Table III) at both 260 and 280 nm and slightly lowers the T_m of free base pairs from 66.5 to 65°. The significant reduction of h_{max} in the complex may imply the existence of another phase or phases of melting which may occur after 117°, the highest temperature examined.

Figure 7 shows the relation between T_m, $T_{m,I}'$, and $T_{m,II}'$ of the complexes and the GC content of DNA used. The average values of T_m, $T_{m,I}'$, and $T_{m,II}'$ are obtained from Tables I–III for different DNA. The results show that the linear relation between melting temperatures and the GC content of DNA (Marmur and Doty, 1962) is obeyed not only for T_m of free base pairs but also for $T_{m,I}'$ and $T_{m,II}'$ of bound base pairs. The slope is the same for $T_{m,I}'$ and $T_{m,II}'$ but is greater for T_m. It is noted that T_m' of polyarginine-copoly(dAT) does not fit the straight line of $T_{m,I}'$ or $T_{m,II}'$. This, perhaps, is due to the fact that copoly(dAT) has its secondary structure different from those of natural DNA as to be reported later (Yu *et al.*, 1974).

Another experiment to examine the two melting bands of polyarginine-bound base pairs is to compare the melting curves at 250 and 280 nm which are respectively more sensitive to AT and GC pairs (Felsenfeld and Sandeen, 1962). The results (not presented here) indicate that the total hyperchromicity of the complex at 280 nm is higher (37.7 for calf thymus and 41.8 for *M. luteus* DNA) than that at 250 nm (30.4 for calf thymus and 20.8 for *M. luteus* DNA). Nevertheless, for each complex, the relative melting area of $T_{m,I}'$ to $T_{m,II}'$ at 280 nm is not different from that at 250 nm. Similar results have also been observed for complexes between polyarginine and *Cl. perfringens* or *E. coli* DNA.

Thermal Denaturation of Poly(Arg^{87}*,Orn*^{13}*)-Calf Thymus DNA Complexes.* The results just presented demonstrate the dependence of melting properties of polyarginine-DNA complexes on GC content and possibly the secondary structure of DNA. Figure 8 further demonstrates the dependence of melting properties of these complexes on polyarginine itself.

TABLE III: Melting Parameters of Polyarginine–Copoly-(dAT) and –Poly(dG)·poly(dC) Complexes.

Synthetic DNA	B (Amino Acid/ Nucleotide)	h_{max}[a] (%)	T_m	T_m'
Copoly(dAT)	0	41.8	23.5	
	0.9	39.7	23.5	72.0
	1.6	36.6	24.0	72.5
Poly(dG)· poly(dC)[b]	0	18.2 (50.8)	66.5	
	0.4	14.1 (31.4)	66.0	
	0.6	9.1 (32.4)	65.0	

[a] See footnote of Table I. [b] Value in parentheses is h_{max} measured at 280 nm.

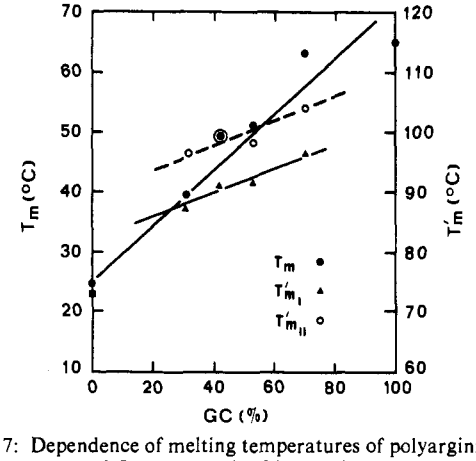

FIGURE 7: Dependence of melting temperatures of polyarginine-DNA complexes on the GC content of DNA. ■ is T_m' for copoly(dAT) which does not fit the straight lines of either $T_{m,I}'$ or $T_{m,II}'$.

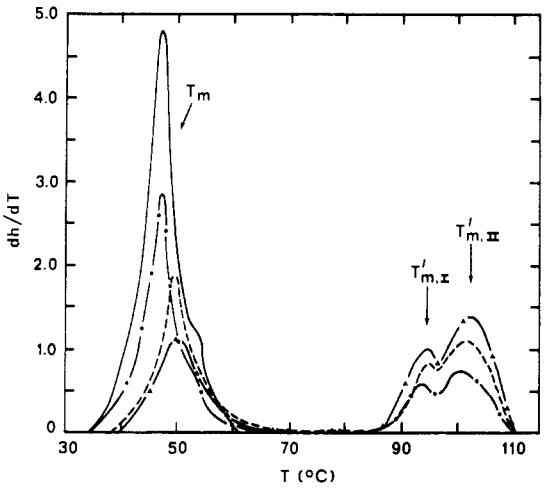

FIGURE 8: Derivative melting profiles of copoly(Arg)^{87}(Orn)^{13}-calf thymus DNA complexes. B = 0 (—), 0.40 (- ● -), 0.60 (- - -), and 0.80 (- ▲ -).

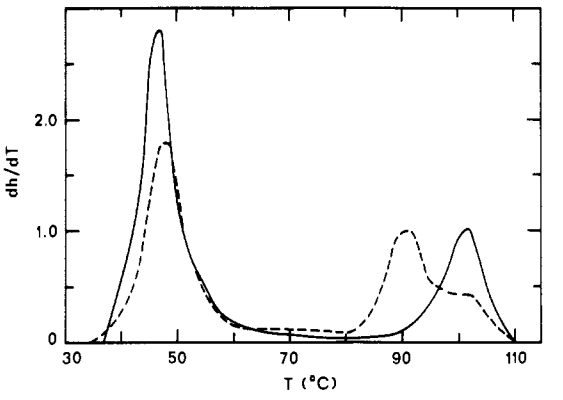

FIGURE 9: Derivative melting profiles of polyornithine-DNA and DNA complexed with a mixture of 87% polyarginine and 13% polyornithine. Polyornithine-DNA with B = 0.3 (——). (87% polyarginine + 13% polyornithine)-DNA with B = 0.4 (- - - -).

When a poly(Arg^{87},Orn^{13}) is used, two melting bands for bound base pairs, $T_{m,I}'$ at 94° and $T_{m,II}'$ at 102°, are also clear. What makes this complex appear differently from that of polyarginine is $A_{T_{m,II}'}/A_{T_{m,I}'}$. This ratio is about 0.45 for calf thymus DNA complexed with polyarginine (Table I) and is about 3.0 for the same DNA but complexed with poly(Arg^{87},Orn^{13}) (Table IV). The increase of this ratio from 0.45 to 3.0 cannot be due to the stabilization of DNA to $T_{m,II}'$ by ornithine as shown in Figure 9. Polyornithine alone stabilizes calf thymus DNA to 102°. A mixture of 87% polyarginine and 13% polyornithine stabilizes DNA to two temperatures, 91 and 102°, respectively. The melting band at $T_{m,I}'$ is still much greater than that at $T_{m,II}'$ which is quite different from the results in Figure 8 when a copolymer of 87% arginine and 13% ornithine is used. Apparently the presence of 13% ornithine in polyarginine changes its binding properties to DNA greatly.

Quantitative Analysis of Thermal Denaturation Results. Let h_b, h_f, A_b, and A_f be respectively the hyperchromicities (h) and the areas of melting bands (A) of base pairs bound by (h_b and A_b) and free of (h_f and A_f) polyarginine or poly(Arg^{87},Orn^{13}). Here we simply use $A_b = A_{T_{m,I}} + A_{T_{m,II}}$ and $A_f = A_T - A_b = h_{max} - A_b$, where A_T is the total area and is equal to the h_{max} of the complex. As defined earlier B is the input ratio of amino acid residue per nucleotide. Define F as the fraction of base pairs bound by polyarginine or poly(Arg^{87},Orn^{13}), the following equations can be used for analyzing the melting data

$$B = \beta F \quad (1)$$

$$B = (\beta/h_b)A_b \quad (2)$$

$$B = \beta[1 - (A_f/h_f)] \quad (3)$$

where β is the average number of amino acid residues per nucleotide in polyarginine- or poly(Arg^{87},Orn^{13})-bound regions (Li *et al.*, 1973) and h_f is the hyperchromicity of free base pairs which is equal to that of pure DNA.

The linear plots of eq 3 and 2 for some complexes are shown in Figures 10 and 11, respectively. Within experimental error these two equations are followed very well. The parameters determined by these plots, namely h_b and β, of each complex, are given in Table V. It is seen from Table V that calf thymus DNA and copoly(dAT) have much higher h_f and h_b than bacterial DNA, *Cl. perfringens*, *E. coli*, and *M. luteus*, though the order of GC content of these DNA follows *M. luteus* > *E. coli* > calf thymus > *Cl. perfringens* > copoly(dAT). Apparently there is no comprehensible relation between the hyperchromicity and the GC content of DNA. The slight variation in h_b for calf thymus DNA complexed with polyarginine (22.5) and with poly(Arg^{87},Orn^{13}) (25.0) can possibly be due to experimental error because h_f for these two experiments also varies slightly from 35.9 to 37.0.

The variation in β, the number of amino acid residues per nucleotide in bound regions, is significant and interesting. For DNA complexed with polyarginine, β varies from 1.90 for copoly(dAT), 1.20 for *Cl. perfringens* DNA, 0.72 for calf thymus DNA, and 0.80 for *E. coli* and *M. luteus* DNA. There is a great dependence of β on the AT content of DNA when the latter reaches a certain level such as 69% in *Cl. perfringens* DNA. For the same calf thymus DNA, β is 0.72 for polyarginine and 1.05 for poly(Arg^{87},Orn^{13}). Apparently the winding of a basic polypeptide with one cation per residue on DNA also depends upon the composition of DNA or the side chains of the amino acid residues.

TABLE IV: Melting Parameters of Poly(Arg^{87},Orn^{13})–Calf Thymus DNA Complexes.

B (Amino Acid/Nucleotide)	h_{max}[a] (%)	T_m	$T_{m,I}'$	$T_{m,II}'$	$A_{T_{m,II}'}/A_{T_{m,I}'}$
0	37.0	47.0			
0.2	35.1	47.5	94.0	102.0	2.9
0.4	31.9	47.0	94.0	101.5	2.4
0.6	30.6	49.0	94.0	101.5	3.8
0.8	30.6	49.5	95.0	102.0	2.8
1.0	27.0	49.5	94.0	102.0	2.9

[a] See footnote of Table I.

Discussion

Though a histone with uneven charge distribution along the molecule can induce two phases of melting when it binds DNA in chromatin ($T_{m,III}$ and $T_{m,IV}$ in Li and Bonner, 1971; Li, 1972; Li *et al.*, 1973), a polylysine with homogeneous charge

TABLE V: Melting Parameters Obtained from Equations 2 and 3.

	Polyarginine–DNA					Poly(Arg^{87},-Orn^{13})–DNA
	Copoly(dAT)	*Cl. perfringens*	Calf Thymus	*E. coli*	*M. luteus*	Calf Thymus
% GC	0	31	42	53	70	42
h_t	41.8	24.8	35.9	27.0	25.3	37.0
h_b	34.6	19.0	22.5	16.5	17.8	25.0
β (amino acid/nucleotide)	1.90	1.20	0.72	0.80	0.80	1.05

distribution along the molecule always induces one phase of melting at T_m' when it binds DNA (Tsuboi *et al.*, 1966; Leng and Felsenfeld, 1966; Olins *et al.*, 1967, 1968; Kawashima *et al.*, 1969; Inoue and Ando, 1970; Shih and Bonner, 1970). A more detailed study on polylysine-DNA complexes shows one symmetric melting band at T_m' for a direct mixed complex (Li *et al.*, 1973) and one asymmetric melting band at T_m' for a reconstituted complex (Li *et al.*, 1974a,b). The latter was attributed to heterogeneous complexes prepared by reconstitution method using NaCl gradient dialysis. In this report, it is shown further that direct-mixed polyarginine-DNA complexes show two melting bands at T_m'. The relative amplitude of these two bands is a function of the GC content of DNA and also of the existence of ornithine in polyarginine. We intend to deal with this problem in the rest of this report.

Theoretical consideration, at least, suggests the following three possibilities for the existence of two melting bands at T_m' for a complex between a polyarginine and a DNA.

(a) Since a free GC pair is thermally more stable than an AT pair (Marmur and Doty, 1962), it is possible that a polyarginine-bound GC pair is also more stable than a bound AT pair, that $T_{m,I}'$ corresponds to bound AT pairs and $T_{m,II}'$ to bound GC pairs. Though GC pairs in pure DNA cannot melt as a distinguishable band from that of AT pairs due to cooperative melting, it is not impossible for this to happen in a polyarginine-bound region as cooperativity of melting is weakened in the complex. If this were the case, one would expect a greater $A_{T_{m,II}'}/A_{T_{m,I}'}$ for DNA with higher GC content. The results in Tables I and II show that this is not the case.

(b) If the binding of a polyarginine on DNA is not identical along the bound DNA segment, it is possible that a portion of the segment is electrostatically less neutralized than the other. Under this condition they may have different thermal stabilities and yield two melting bands ($T_{m,I}'$ and $T_{m,II}'$).

Previously it was shown that those two melting bands of histone-bound regions, $T_{m,III}$ at 66° and $T_{m,IV}$ at 82°, in pea bud nucleohistone (Li and Bonner, 1971) correspond to bound base pairs with different electrostatic shielding which respond differently to ionic strength in solution. This concept is the basis for suggestion (b). Contrary to expectation from the results in nucleohistone, Figure 3 shows no shift of $T_{m,I}'$ and $T_{m,II}'$ as the ionic strength of the solution is increased. Of course, there is some possibility that the dependence of melting temperature of a protein-bound region on ionic strength may be reduced to a negligible level as the ionic neutralization on phosphate lattice by protein reaches a certain point. If this is the case the results in Figure 4, still, cannot be used to support or rule out suggestion (b). However, the β values in Table V are useful for this purpose. If suggestion (b) were correct, a complex with greater melting band at $T_{m,II}'$ would have more arginine per nucleotide or greater β value. The results in Table V show the opposite, namely 0.72 for calf thymus DNA and 0.8 for *E. coli* and *M. luteus* DNA which disfavor this suggestion.

(c) When a polyarginine is added to DNA it can wind along either the major or the minor groove of DNA. DNA segments bound by the same polyarginine but in opposite grooves have different melting temperatures at $T_{m,I}'$ and $T_{m,II}'$. The probability for polyarginine to wind along either groove is a function of the GC content and the conformation of DNA.

Several factors can contribute to thermal stability of a protein bound-DNA segment, such as AT or GC content, charge neutralization on phosphates, hydration in the grooves, and interaction between amino acid residues and nucleotides. The

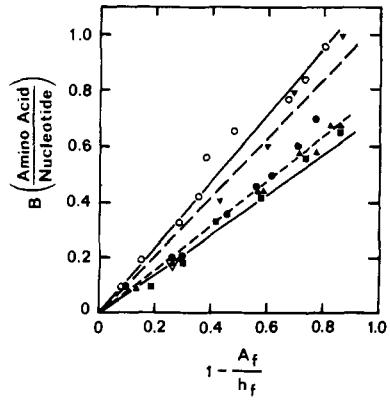

FIGURE 10: Linear plot of eq 3. Polyarginine complexed with DNA from *Cl. perfringens* (○ ——○), calf thymus (——), *E. coli* (▲ - - -▲), and *M. luteus* (● - - - ●). Poly(Arg^{87},Orn^{13}) with calf thymus DNA (▼ - - -▼).

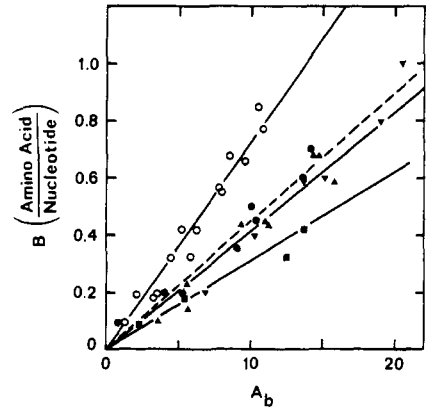

FIGURE 11: Linear plot of eq 2. Polyarginine complexed with DNA from *Cl. perfringens* (○ —○), calf thymus (■ ——■), *E. coli* (▲ — — ▲), and *M. luteus* (○ - - - ○). Poly(Arg^{87},Orn^{13}) with calf thymus DNA (▼——▼).

first two factors have just been excluded as causes for $T_{m,I}'$ and $T_{m,II}'$ in polyarginine–DNA complexes. Dehydration has been considered as the cause for conformation change from B to C form in DNA (Tunis-Schneider and Maestre, 1970; Nelson and Johnson, 1970; Li *et al.*, 1971) and also its stability (Tunis and Hearst, 1968). It is likely that, quantitatively, hydration in either groove can have a different effect in maintaining DNA structure and its stability. In addition to the possible difference in hydration in either groove due to polyarginine binding, the possible interaction between arginine residue and nucleotide can also lead to differential thermal stability when this interaction occurs either in the major or minor groove. Therefore, it is not unreasonable to assume that $T_{m,I}'$ and $T_{m,II}'$ in polyarginine–DNA complexes are due to the melting of DNA segments bound by polyarginine in opposite grooves. To which groove does $T_{m,I}'$ or $T_{m,II}'$ correspond is still not clear.

Two factors should be considered for a basic polypeptide to wind along either groove of DNA. As the backbone of a polypeptide winds along the groove the side chain extends to the phosphate (Tsuboi *et al.*, 1966); the length of amino acid side chain and the secondary structure of DNA can be critical for the selection of the groove for protein binding. The molecular environments in the grooves are different; if interaction between amino acid residues and nucleotides (particularly on the bases) plays an important role in the binding between protein and DNA, the chemical property of the amino acid side chain and the AT or GC content of DNA will also become critical for the selection of the groove for protein binding.

The above two factors fit the experimental results very well. For instance, the length of side chain of an arginine is greater than lysine than ornithine. The side chain of arginine is also quite different from those of lysine and ornithine because of its guanido group. The experimental results show that the presence of 13% of ornithine in polyarginine shifts the major melting from $T_{m,I}'$ to $T_{m,II}'$. The latter, at about 100–102°, is closer to the T_m' at 99–101° for polylysine-bound base pairs (Li *et al.*, 1973). This result is in agreement with the expectation that the selection of the groove for protein binding depends upon the side chain of the amino acid residues. It also agrees with the fact that the side chain of ornithine is chemically closer to lysine than to arginine. The dependence of $A_{T_{m,II}'}/A_{T_{m,I}'}$ on the GC content of DNA is also in agreement with the above discussion.

It is possible that specific binding of polyarginine to DNA depends upon the secondary structure of the latter though it is double-stranded. For instance, copoly (dAT) has a D conformation which is different from the B conformation of natural DNA (Davies and Baldwin, 1963). The CD spectrum of this copoly (dAT) is also quite different from those of natural DNA (Yu *et al.*, 1974). In those complexes with polyarginine, copoly (dAT) shows only one T_m' not two, and its β value (1.9) is also much greater than those of natural DNA (0.7–1.2). Apparently the winding of polyarginine on copoly(dAT) is quite different from that on natural DNA.

If the suggestion that polyarginine can bind DNA in either groove with $T_{m,I}'$ or $T_{m,II}'$ is accepted as a tentative conclusion, the question regarding whether polyarginine stabilizes DNA less than that by polylysine (Yu and Li, 1973) will depend upon whether polyarginine binds DNA in the same groove as that by polylysine or not.

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