Studies on Interaction between Histone V (f2c) and Deoxyribonucleic Acids†

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ABSTRACT: Histone V (f2c) from chick erythrocytes was used in the study of its interaction with DNA from various sources. Complexes between this histone and DNA were formed using the procedure of continuous NaCl gradient dialysis in urea. Two physical methods, namely thermal denaturation and circular dichroism (CD), were used as analytical tools. Thermal denaturation of nucleohistone V with chick or calf thymus DNA shows three melting bands: band I at 45–50° corresponds to free base pairs; band II at 75–79°, and band III at 90–93° correspond to histone-bound base pairs. In histone-bound regions, there are 1.5 amino acid residues/nucleotide in nucleohistone V. In contrast, a value between 2.9 and 3.3 was determined for nucleohistone I (f1) (H. J. Li (1973), *Biopolymers 12,* 287). Similar melting properties have been observed for histone V complexed with bacterial DNA from *Micrococcus luteus*. Histone V binding to DNA induces a slight transition from a B-type CD spectrum to a C-type spectrum. Trypsin treatment of nucleohistone V reduces melting band III much more effectively than band II. Such a treatment also restores DNA to B conformation in the free state. Reduction of the melting bands of nucleohistone V by polylysine binding follows the order of I > II > III, accompanied by the increase of a new band at 100°. When two bacterial DNAs of varied A + T (adenine + thymine) content simultaneously compete for the binding of histone V, the more (A + T)-rich DNA is selectively favored. Under experimental conditions described here, *Clostridium perfringens* DNA with 69% A + T is bound by histone V in preference to chicken DNA with 56% A + T although the latter has natural sequences for histone V binding.

Among the chromosomal proteins, it has been widely assumed that histones play structural roles and non-histone proteins regulatory roles. Histones have been well characterized in terms of composition and sequence (DeLange et al., 1969, 1973; Ogawa et al., 1969; Iwai et al., 1970; Rall and Cole, 1971; Sautiere et al., 1972; Yeoman et al., 1972) and generally have been considered to lack species and tissue specificity (Fambrough and Bonner, 1968; DeLange et al., 1969; DeLange and Smith, 1971). A close examination of the existing data on individual histones, however, reveals that both of these statements are over-simplified. Enzymic modification of histones during the cell cycle and subsequent development have been observed while a definite relation between this modification and function has not yet been assigned. Histone V (f2c) is known to be tissue specific, in that it is found almost exclusively in nucleated erythrocytes (Neelin et al., 1964; Hnilica, 1964, 1972; Champagne and Mazen, 1967). The relative inactivity of these erythrocyte nuclei, when correlated with the appearance of histone V, implies a repressor role for this histone. Consequently there is particular importance of studying physical interaction between this histone and DNA.

In this communication the interaction between histone V from chicken erythrocytes and DNA from chicken, calf thymus, *Clostridium perfringens,* and *Micrococcus luteus* has been studied with an aim to learning the stoichiometry and sequence specificity in binding, as well as the thermal stabilization and conformational effect on DNA when bound by this histone. Results from these studies are compared with those of DNA complexed with other histones in chromatin and in reconstituted nucleohistone.

Materials and Methods

Erythrocytes were obtained from freshly sacrificed adult Leghorn chicken. Histones I (f1) and V (f2c) were selectively extracted from chromatin (Champagne and Mazen, 1967) which were isolated from the nuclei of erythrocyte cells. Histone V was then separated from histone I by amberlite CG50 chromatography eluted by guanidinium chloride at 17.5% (Adams and Neelin, 1969; Mura et al., 1974). Purity of the sample was assayed by the gel electrophoresis system of Panyim and Chalkley (1969) and by immunoassay (Mura et al., 1974).

Calf thymus DNA was purchased from Sigma Chemical Co. and was purified by phenol extraction. Chicken DNA was prepared by the method of Marmur (1961) from chicken erythrocyte chromatin. *Cl. perfringens* DNA from Sigma Chemical Co., and *M. luteus* DNA from Miles Laboratories were used directly without further purification. At 260 nm the molar extinction coefficient of DNA used was 7400 M^{-1} cm^{-1} for *Cl. perfringens,* 7000 for *M. luteus* (Felsenfeld and Hirschman, 1965), and 6500 for calf thymus and chicken.

Reconstituted nucleohistone V was prepared by continuous NaCl gradient dialysis in decreasing concentrations from 2.0 *M* to about 0.1 *M* in the presence of 5 *M* urea–0.01 *M* Tris (pH 8.0) over a period of about 48 hr (Li and Bonner, 1971). This method is a modification of the one first used by Huang and Huang (1969) and Bekhor et al. (1969). Urea was then dialyzed out in the presence of 0.015 *M* NaCl–0.01 *M* Tris (pH 8.0). The complexes were finally dialyzed against 2.5×10^{-4} *M* EDTA (pH 8.0) (EDTA buffer) for CD and thermal denaturation measurements.

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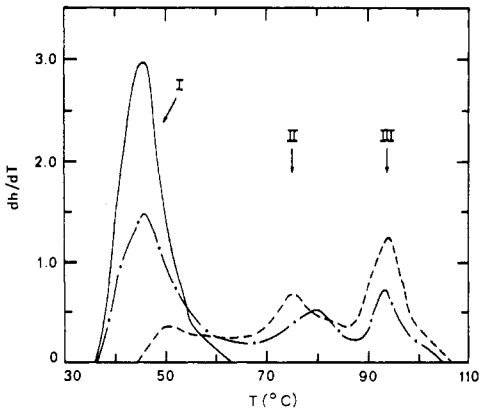

FIGURE 1: Derivative melting profiles of histone V-chicken DNA complexes. r = 0 (—), 0.5 (- · -), and 1.0 (- - -).

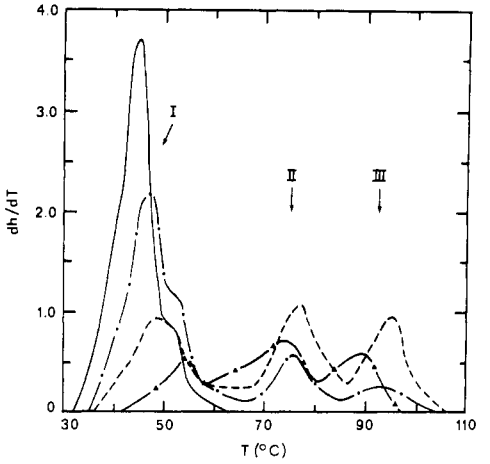

FIGURE 2: Derivative melting profiles of histone V-calf thymus DNA complexes. r = 0 (—), 0.5 (- · -), 1.0 (- - -), and 1.5 (-▲-).

A molar extinction coefficient of 4.7×10^2 M^{-1} cm^{-1} at 230 nm has been used before for histones, where M refers to moles/liter of amino acid residues (Ohlenbusch et al., 1967). The validity of this value was confirmed for histone V by Lowry's determination (Lowry et al., 1951). After the concentration in water was determined, it was added with salt and urea solution to a final concentration of 2.0 M NaCl-5 M urea-0.01 M Tris (pH 8.0) for complex formation. For nucleohistone V molar extinction coefficient of 6500 m^- cm^{-1} at 260 nm was used. The contribution of light scattering to the absorbance at 260 nm was corrected according to Leach and Scheraga (1960). For each nucleohistone V, the input ratio of histone to DNA, r, is reported as amino acid residues per nucleotide.

For proteolytic digestion of nucleohistone V, salt-free crystals of trypsin (A grade) from Calbiochem were used. The trypsin was prepared at a concentration of 0.1 mg/ml and dialyzed against EDTA buffer before use. Trypsin inhibitor from Soybean (type 1-S) was purchased from Sigma Chemical Co. An appropriate amount of trypsin was added to nucleohistone V at room temperature, and the final concentration of trypsin was recorded. An aliquot of 0.02 ml of trypsin inhibitor (1 mg/ml) was added to the mixture at 0.5 hr or 1.5 hr after the reaction was started. The samples were then examined by CD and thermal denaturation.

Poly(L-lysine) hydrochloride (mol wt 15,500) was purchased from Schwartz/Mann. Polylysine-nucleohistone V complexes were made by direct mixing as described earlier (Li et al., 1972). The buffer used was 2.5×10^{-4} M EDTA (pH 8.0).

All absorbance and changes in absorbance during thermal denaturation were measured using a Gilford spectrophotometer, Model 2400-S, coupled to a temperature regulator. dh/dT is used here to describe the melting profile, where h is the percent increase in absorbance with heating when referred to the absorbance A_{260} at room temperature and T is the temperature in °C.

The CD spectra of the samples were taken on a Jasco spectropolarimeter, Model J-20, at room temperature. $\Delta\epsilon = \epsilon_L - \epsilon_R$ is reported, where ϵ_L and ϵ_R are respectively molar extinction coefficients for the left- and the right-handed circularly polarized light. The units of $\Delta\epsilon$ are M^- cm^{-1} in terms of nucleotide.

Results

Thermal Denaturation of Nucleohistone V. Figure 1 shows typical derivative plots of melting profiles of histone V-chicken DNA complexes. Pure chicken DNA has a critical melting temperature (T_m) at 45° with a shoulder at about 56°. A similar shoulder was observed in calf thymus DNA and interpreted as a more (G + C)-rich fraction in a heterogeneous population of DNA molecules, possibly the satellite fraction (Li et al., 1974a). As increasing amounts of histone V are complexed to DNA, two additional melting bands are observed. Melting band I of free DNA is proportionally reduced with a corresponding increase of the two new melting bands, band II at 75-79° and band III at 90-93°. These two melting bands, corresponding to base pairs bound by histone V, are similar to those found in the same buffer in pea bud chromatin (Li and Bonner, 1971), in calf thymus chromatin (Li et al., 1973), in reconstituted nucleohistone (IIb1 + IIb2) (f2a2 + f2b) (Li and Bonner, 1971), and in nucleohistone IIb2 (f2b) (Leffak et al., 1974), except that in nucleohistone V these two melting temperatures are 5-10° higher. Other histone-DNA complexes prepared by direct mixing in urea also have been reported to show three melting bands, two of which are attributable to histone binding (Ansevin and Brown, 1971).

In order to test whether the characteristic melting properties of nucleohistone V depend upon the specific base sequence of chicken DNA, calf thymus DNA was used for complex formation with histone V. The melting results of these complexes are shown in Figure 2 and are essentially the same as those in Figure 1 when chicken DNA was used. It is interesting to note that as more histone V is added to calf thymus DNA, the major melting band near 45° is greatly reduced, but the shoulder near 52° is not affected, a phenomenon also observed in reconstituted polylysine-calf thymus DNA complexes. In the latter case the observation was explained as a selective binding of polylysine to the more (A + T)-rich fraction of a heterogeneous DNA population (Li et al., 1974a). As will be shown later, this interpretation can be applied to histone V binding to DNA.

Although the same melting properties are observed for complexes between chicken histone V and DNA from either chicken or calf thymus, it still could be argued that both DNAs are from eukaryotic tissues and would be expected to bind histone V in the same manner. In order to test this possibility, prokaryotic *M. luteus* DNA was used. As shown in Figure 3, histone V-*M. luteus* DNA complexes also show three melting bands similar to those in Figure 1 and 2 when chicken and calf thymus DNAs were used, except that the melting temperatures of these three bands are higher for *M. luteus* complexes than are the corresponding ones for chick-

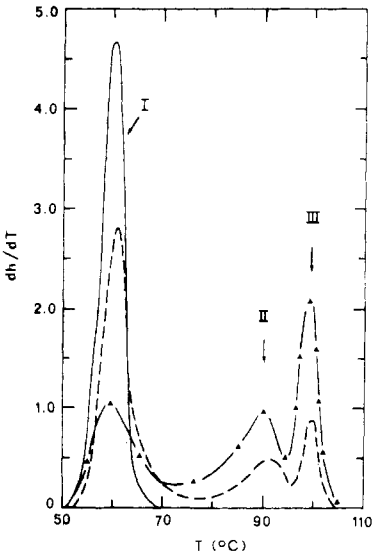

FIGURE 3: Derivative melting profiles of histone V-*M. luteus* DNA complexes. $r = 0$ (—), 0.5 (- - -), and 1.0 (-▲-).

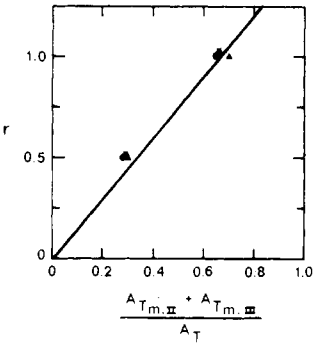

FIGURE 4: Linear plot of eq 1. Chicken DNA (▲), calf thymus DNA (●), and *M. luteus* DNA (■).

en and calf thymus nucleohistone V. Based upon prior studies this is expected because G + C content for *M. luteus* DNA is higher than those for chicken and cow. For instance, it has been shown earlier that when DNA base pairs are bound by polyarginine (Epstein et al., 1974) or by polylysine (Li et al., 1975) the melting temperature is higher for bound G · C pair than for A · T, a finding which has proved true for pure DNA base pairs as well (Marmur and Doty, 1962).

For both chicken and calf thymus DNAs, when r, the input ratio of histone to DNA in reconstituted nucleohistone V, was equal to or greater than 1.5 amino acid residues/nucleotide, significant amounts of insoluble precipitates were detected. In most cases, the absorbance in the solution was too low for any meaningful measurement. One melting curve of the complex with $r = 1.5$ is included in Figure 2. In this sample the area of the melting band at 55° is still about 20% of the total area under the whole melting curve. The two melting bands, II and III, of histone-bound base pairs of this complex are also about 3–5° lower than the corresponding bands from complexes of lower r values.

As has been proposed earlier (Li, 1973), melting data can be used for evaluating the ratio of amino acid residues per nucleotide (β) in the histone-bound regions of nucleohistone using

$$r = \beta(A_{T_{m,II}} + A_{T_{m,III}})/A_T \qquad (1)$$

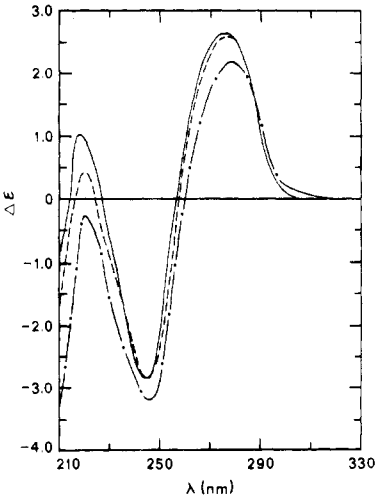

FIGURE 5: CD spectra of histone V-chicken DNA complexes. $r = 0$ (—), 0.5 (- - -), and 1.0 (- · -).

where $A_{T_{m,II}}$ and $A_{T_{m,III}}$ are the melting areas under bands II and III, respectively, and A_T the total melting area which is equal to h_{max}.

Results derived from eq 1 for histone V complexed with DNA from chicken, calf thymus, or *M. luteus* are shown in Figure 4. β is 1.5 amino acid residues/nucleotide for all three DNAs. Quantitatively the melting properties of histone V complexed with DNA from eukaryotic chicken or cow, or prokaryotic *M. luteus*, are identical with one another. The value of 1.5 amino acids/nucleotide in nucleohistone V is much lower than the 3.0–3.5 obtained with chromatin or 2.9–3.3 with nucleohistone I (Li, 1973; Li et al., 1973).

Circular Dichroism of Nucleohistone V. Conformation of free histone V in solution has been studied by nuclear magnetic resonance (Bradbury et al., 1972) and CD (Williams et al., 1972; Williams and Seligy, 1973). Here we report CD studies of the conformation of histone V-chicken DNA complexes. The results are shown in Figure 5. As more histone V is complexed to the DNA, there are slight red shifts for both the positive band at 275 nm (λ_{max}) and the crossover at 256 nm (λ_c) and a reduction of $\Delta\epsilon_{278}/-\Delta\epsilon_{246}$ ratio. Histone V in the chicken DNA complex also makes a negative CD contribution near 220 nm. Similar CD results were observed for histone V-calf thymus DNA complexes.

Based upon the red shift for λ_{max} and λ_c and the reduction of $\Delta\epsilon_{278}/-\Delta\epsilon_{246}$, histone V binding to DNA seems to induce a transition from a B-type CD spectrum to a C-type spectrum. This could possibly indicate an induced conformational transition in DNA from B toward C structure. The degree of distortion of the CD spectrum of DNA induced by histone V binding is small when compared with that induced by protamine (Yu and Li, 1973), histone IIb2 (Leffak et al., 1974), polylysine (Chang et al., 1973), or polyarginine (Yu et al., 1974).

Trypsin Digestion of Nucleohistone V. Adding trypsin to calf thymus chromatin reduces the highest melting band before affecting the second highest band (Ansevin et al., 1971; Li et al., 1974b); these bands correspond respectively to melting bands III and II in this report. Similar results have been observed for nucleohistone V (Figure 6). When 5 μg/ml of trypsin is added to histone V-chicken DNA complex with $r = 1.0$ for 0.5 hr before the addition of inhibitor,

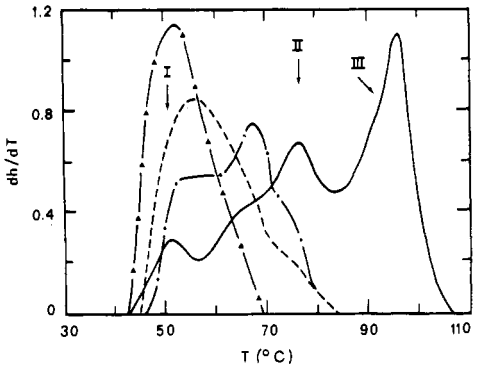

FIGURE 6: Effect of trypsin treatment on derivative melting profile of histone V-chicken DNA complex (r = 1.0). Control (—), trypsin treatment with 5 μg/ml for 0.5 hr (- · -), 20 μg/ml for 0.5 hr (- - -), and 20 μg/ml for 1.5 hr (-▲-).

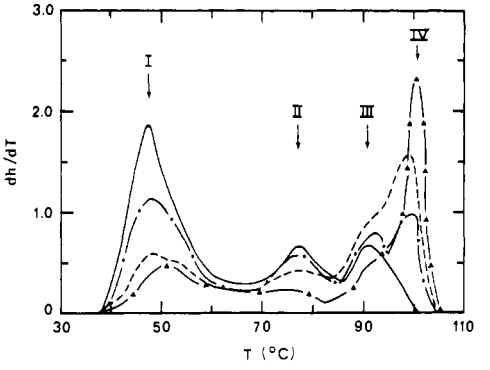

FIGURE 7: Effect of polylysine binding on derivative melting profile of histone V-calf thymus DNA complex (r = 0.5). Input lysine/nucleotide is 0 (—), 0.2 (- · -), 0.4 (- - -), and 0.5 (-▲-).

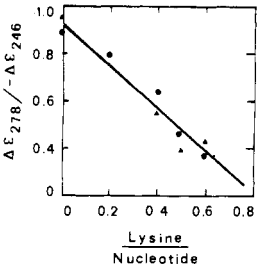

FIGURE 8: Comparison of effect of polylysine binding on CD spectrum of DNA and histone V-DNA complex. Calf thymus DNA (▲), histone V-calf thymus DNA complex with r = 0.5 (●).

melting band III disappears completely. Melting band II is substantially reduced with the appearance of more melting between 50 and 70°. Treatment of nucleohistone V with 20 μg/ml of trypsin for 0.5 hr reduces melting band II further, accompanied by an increase of melting at about 57°. The melting near 70° disappears completely when 20 μg/ml of trypsin is added to nucleohistone V for a 1.5 hr period before adding the inhibitor. Under this last condition, a significant amount of melting is also shifted from 57 to 51°.

Trypsin digestion of nucleohistone V causes a slight blue shift in both λ_{max} and λ_c of the DNA CD spectrum and reduces the negative CD at 220 nm originally contributed by bound histone V. It can be inferred that the bound histone V was digested, thus freeing the DNA from distortion and restoring its conformation toward that of pure DNA. Trypsin-treated histone V-calf thymus DNA complexes yield thermal denaturation and CD results similar to those of histone V-chicken DNA.

Polylysine Binding to Nucleohistone V. Polylysine binding to calf thymus chromatin has been shown to reduce not only the area of melting bands of histone-free regions but also that of the second highest melting band. The latter was attributed to base pairs bound by the less-basic halves of histones (Li et al., 1972, 1973). In order to see whether or not similar results could be produced in a system reconstituted from pure individual histones and DNA, experiments were done in which polylysine was bound to histone V-calf thymus DNA complex with r = 0.5. Figure 7 shows the results of thermal denaturation. As polylysine is added to nucleohistone V, melting band I is greatly reduced; there is a slight decrease of band II and an accompanying increase in a new band IV appearing at 98–100°. This phenomenon was also observed with nucleohistone and NaCl-treated nucleohistones from calf thymus bound by polylysine (Li et al., 1972, 1973). Because of a significant overlap between melting bands III and IV and a slight shift of $T_{m,IV}$ at various input ratios of polylysine to nucleohistone, an accurate evaluation of the effect on melting band III by polylysine binding becomes difficult. Nevertheless, from Figure 7, it can be deduced that the reduction in the amplitude of melting bands in nucleohistone V caused by polylysine binding follows the order: band I > II > III. This preferential sequence is the same as that found in polylysine binding of calf thymus nucleohistone (Li et al., 1973).

The CD effects on nucleohistone V induced by polylysine binding are similar to those found in complexes of polylysine and pure DNA (Chang et al., 1973). In order to compare the CD results of polylysine–nucleohistone V and polylysine–DNA complexes, the CD ratio, $\Delta\epsilon_{278}/-\Delta\epsilon_{246}$ is used. Figure 8 shows the dependence $\Delta\epsilon_{278}/-\Delta\epsilon_{246}$ on the input ratio of polylysine to DNA or nucleohistone V. Within the accuracy of measurement, there is no significant difference between these two systems.

Both thermal denaturation and CD measurements were also made on polylysine–histone V-chicken DNA complexes. The results and conclusions are similar to those shown in Figures 7 and 8 from polylysine–histone V-calf thymus DNA complexes.

Selective Binding of Histone V to (A + T)-Rich DNA. Leng and Felsenfeld (1966) showed that at 1.0 *M* NaCl, polylysine favored (A + T)-rich DNA for binding. Clark and Felsenfeld (1972) also reported that arginine-rich histone-bound regions in chromatin were slightly (G + C) rich. In order to test whether histone V favors (A + T)- or (G + C)-rich DNA for binding under the experimental condition of reconstitution by NaCl gradient dialysis with urea, a mixture of equimolar *Cl. perfringens* (69% A + T) and *M. luteus* (30% A + T) DNA was used as a starting material for making reconstituted nucleohistone V. The results in Figure 9 show that at r = 0, there are two melting bands at 40.5 and 61.5° corresponding to the melting of *Cl. perfringens* and *M. luteus* DNA respectively. At r = 0.5, the melting band of free *Cl. perfringens* is greatly reduced, but not that of *M. luteus*, and there is a corresponding increase in hyperchromicity at higher temperature. At r = 1.0, the *Cl. perfringens* band disappears entirely, and the *M. luteus* band is reduced by about 50%. These results are similar to those observed for competitive binding of polylysine, with its selective binding to (A + T)-rich DNA during salt gradient dialysis (Li et al., 1974a). Therefore, it is concluded that under the experimental conditions of reconstitution, histone V binds selectively to (A + T)-rich DNA.

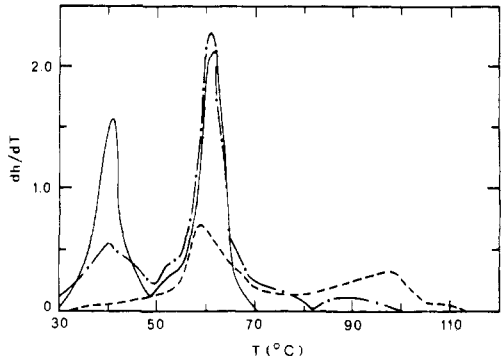

FIGURE 9: Selective binding of histone V to (A + T)-rich DNA. An equimolar mixture of *Cl. perfringens* DNA and *M. luteus* DNA was complexed with histone V by reconstitution. r = 0 (—), 0.5 (- · -), and 1.0 (- - -).

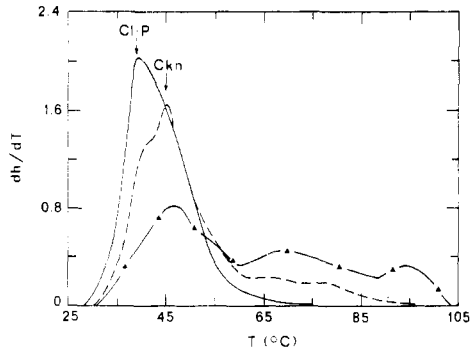

FIGURE 10: Competition between chicken and *Cl. perfringens* DNA for histone V binding. The arrows indicate the melting temperatures for chicken and *Cl. perfringens* DNA. An equimolar mixture of chicken and *Cl. perfringens* DNA was complexed with histone V by reconstitution. r = 0 (—), 0.5 (- - -), and 1.0 (-▲-).

Importance of A + T Content vs. Base Sequence in DNA for Histone V Binding. Both *Cl. perfringens* and *M. luteus* are bacteria. DNA from these prokaryotes, which do not contain histones, is not expected to carry any specific sequences for histones, while such sequences exist in eukaryotic DNA where histones are found. The results in Figure 9 indicate that when two bacterial DNAs without any natural base sequence for histones compete for histone V binding, (A + T)-rich DNA is strongly favored over (G + C)-rich DNA. In order to see whether this A · T preference or the native base sequence in a eukaryotic DNA is more important for histone V binding, a mixture of equimolar *Cl. perfringens* (69% A + T) and chicken DNA (56% A + T) was used. The former has a slightly higher A + T content, while the latter has natural sequences for histone V. Since the percent of A + T content of these two DNAs is close, their melting curves overlap and a broad melting band results, as shown in Figure 10. When these two DNAs melt separately, the T_m is 39° for *Cl. perfringens* and 45° for chicken. When histone V is bound to this mixture of DNA, a greater amount of melting is reduced at the lower temperature side of this band accompanied by an increase of melting bands above 60°. The results become clearer if the differences of derivative melting curves, $\Delta(dh/dT) = (dh/dT)_{complex} - (dh/dT)_{DNA}$, are taken (Figure 11). The negative peak of the curve is located at 39 to 40°, the melting temperature of *Cl. perfringens* DNA. Since this is the temperature most affected by the histone V binding, it appears that *Cl. perfringens* DNA is a much stronger competitor than chicken

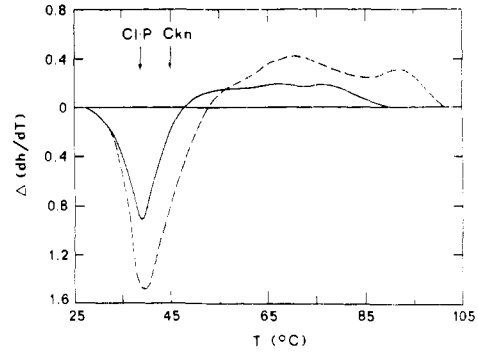

FIGURE 11: Difference derivative melting profiles of Figure 10. $\Delta(dh/dT) = (dh/dT)_{complex} - (dh/dT)_{DNA}$. For the complex, r = 0.5 (—) and 1.0 (- - -).

DNA for histone V. In other words, under the experimental condition described, the affinity for histone V is primarily determined by A + T content rather than by the base sequence.

Discussion

Based upon thermal stabilization and conformational effect on DNA due to histone binding, the results from this study show a strong and direct interaction between this histone and DNA. This implies that a strong and direct interaction probably could occur in chromatin.

The two melting bands observed with nucleohistone V in EDTA buffer are similar to those reported earlier with pea bud chromatin and reconstituted nucleohistone (IIb1 + IIb2) (Li and Bonner, 1971), as well as with calf thymus chromatin (Li et al., 1973) and reconstituted nucleohistone IIb2 (Leffak et al., 1974), except that in nucleohistone V the melting temperatures are higher. This perhaps can be correlated with a higher content of positively charged basic amino acid residues in chicken histone V (32–40%) than in any other histone from either cow or pea (25–30%) (Hnilica, 1972). The presence of two phases of melting induced by histone binding has been interpreted as resulting from uneven charged neutralization on the phosphate lattice of DNA due to the binding of histone molecules which have unevenly distributed basic amino acid residues (Li and Bonner, 1971; Li et al., 1973). Such uneven distribution of basic residues along histone molecules has been shown to be universal among all the common histones (DeLange and Smith, 1971; Hnilica, 1972), such as in histone IV (f2a1) (DeLange et al., 1969; Ogawa et al., 1969), in histone IIb2 (f2b) (Iwai et al., 1970), histone I (f1) (Rall and Cole, 1971), histone III (f3) (DeLange et al., 1970), and histone IIb1 (f2a2) (Sautiere et al., 1972; Yeoman et al., 1972). The two phases of melting in DNA induced by histone V binding are probably subject to the same interpretation. So far, partial sequence data of histone V (Cieplinski, 1971; Greenaway and Murray, 1971; Greenaway, 1971; Freedlander and Smithies, 1974 personal communication) have not been sufficiently revealing to allow a definitive conclusion.

With respect to trypsin digestion, at a higher trypsin level there is more reduction of melting band III than of band II, with a resultant increase in melting band I as shown in Figure 6. Similar results have been noted in calf thymus chromatin (Ansevin et al., 1971; Li et al., 1974b). In the latter case, the melting results were explained as due to a more favorable digestion of the more basic regions of histones by

trypsin than the less basic regions (Li et al., 1973). This hypothesis has recently been verified by Weintraub and Van Lente (1974) who showed that in chromatin histones IIb1, IIb2, III, and IV are cleaved by trypsin at the N-termini (the more basic regions) while histone I and V are attacked all over their molecules. Weintraub and Van Lente's findings could be used to explain the quantitative differences in the results of trypsin digestion of chromatin and reconstituted nucleohistone V. With 20 μg/ml of trypsin reaction for a 1.5-hr period before adding trypsin inhibitor, both melting bands III and II of nucleohistone V are completely eliminated, while under the same experimental conditions, only the highest melting band in calf thymus chromatin is completely eliminated and the one just below is not reduced at all (Li et al., 1974b, 1975a). In other words, the second highest melting band is well protected from trypsin action in calf thymus chromatin but not in nucleohistone V. It is possible that this is a result of less protective α-helical structure in the bound histone V of this nucleohistone than in the bound histones of calf thymus chromatin. For example, a big negative CD at 220 nm has been observed in chromatin (Shih and Fasman, 1970; Simpson and Sober, 1970; Permogorov et al., 1970; Johnson et al., 1972; Chang and Li, 1974; Wilhelm et al., 1974) which is assumed to have considerable α-helical structure, but there is no big negative $\Delta\epsilon_{220}$ in reconstituted nucleohistone V (Figure 5). In addition, the lysine and arginine content of histone V is higher than that of histones in calf thymus chromatin and this could contribute to the higher vulnerability of bound histone V to trypsin. Of course, it is also possible that the binding of histones to DNA in calf thymus chromatin is different from that of histone V to DNA in nucleohistone V such that they respond differently to trypsin digestion.

Recently, using electron microscopy, Olins and Olins (1974) reported a model with particulate structures of chromatin which seems to contradict the model with supercoiled structures of chromatin revealed by X-ray diffraction (Pardon et al., 1967). Based upon thermal denaturation and CD results of native and NaCl-treated chromatin, trypsin-treated chromatin, and polylysine-bound chromatin, and other results in literature, Li et al. (1975a) proposed a model that chromatin molecule is composed of condensed and extended regions: the condensed regions correspond to DNA segments bound by the subunits of whole histones minus histone I and have more α-helical structures with respect to histones and more base tilting with respect to DNA; the more extended regions correspond to free DNA segments or DNA segments bound by histone I or non-histone proteins and have less α-helical structures with respect to proteins and less base tilting with respect to DNA. This model could explain both particulate model of Olins and Olins (1974) and supercoil model of Pardon et al. (1967). In addition, it was suggested there that histone I with little α-helical structure might not protect DNA from nuclease digestion as well as did other histones. With this suggestion, 50% of nuclease-resistant regions in chromatin (Clark and Felsenfeld, 1972) agrees with 50–60% of histone-protected regions in histone I-deficient chromatin (0.6 *M* NaCl-treated chromatin) as determined by thermal denaturation (Li et al., 1973).

It is interesting to note that in histone-bound regions of reconstituted nucleohistone V there are only 1.5 amino acid residues/nucleotide. This value is much lower than the 3.2 of pea bud chromatin (Li, 1973), 3.7 of calf thymus chromatin (Li et al., 1973), and 3.0 of reconstituted nucleohistone I (Li, 1973; Olins, 1969; Shih and Bonner, 1970). If histone V binds DNA in erythrocyte chromatin in a manner similar to that in reconstituted nucleohistone V, this lower figure might imply that histone V suppresses the genome much more effectively than do other histones, because the same amount of histone V can block a longer sequence of DNA genome than the others.

Histone V binding to DNA yields the same quantitative melting results irrespective of the DNA used, whether from chicken, from cow, or from *M. luteus*. This fact could possibly indicate that the specific type of binding between histone V and DNA is primarily determined by the histone itself, rather than by the A + T content or the base sequence in a DNA molecule.

In 1.0 *M* NaCl, or during salt gradient dialysis, polylysine binds selectively to (A + T)-rich DNA (Leng and Felsenfeld, 1966; Li et al., 1974a). The results in Figure 9 show that histone V also preferentially selects A · T pairs for binding. Such selectivity for A · T pairs might possibly be due to the presence of a higher percentage of lysine residues in histone V; it is also possible that the presence of other amino acid residues and different secondary structures in histones could be determining factors. In addition, the experimental conditions used for complex formation could influence the selectivity. Selective binding to (A + T)-or (G + C)-rich DNA by different histones, and the factors contributing to it, are currently under investigation in our laboratory.

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