Histone·Deoxyribonucleic Acid Complexes Studied by Thermal Denaturation and Circular Dichroism Spectroscopy†

I. Michael Leffak‡ and Hsueh Jei Li*,§

ABSTRACT: In order to understand the nature of histone-DNA and histone-histone interaction in reconstituted systems, an extensive study has been made on complexes of total calf thymus histones, histones (H2A + H2B + H3 + H4), (H2A + H2B), H2A, or H2B, with DNA purified from calf thymus with 42% (guanine + cytosine) or G + C, *Micrococcus luteus* with 70% G + C or *Clostridium perfringens* with 31% G + C. These complexes were prepared by one to several of the following five methods: (a) NaCl gradient dialysis in urea, (b) NaCl gradient dialysis without urea, (c) direct mixing in 0.25 mM EDTA, pH 8.0 (EDTA buffer), (d) direct mixing in 0.15 M NaCl, 0.25 mM EDTA, pH 8.0, or (e) direct mixing in 0.01 M sodium phosphate, pH 7.0. All complexes were dialyzed to 0.25 mM EDTA, pH 8.0, for measurements. They were examined by thermal denaturation and circular dichroism (CD) spectroscopy. Thermal denaturation measurements examine both the quality of binding to DNA by each half of the histone molecule and the average length of DNA bound by each molecule in the histone·DNA complex. CD measurements, on the other hand, evaluate the secondary structure of the histones and the conformational effect on the DNA induced by histone binding. Using as reference the properties of chromatin determined by both thermal denaturation and CD, the quality of a reconstituted histone·DNA complex can be evaluated. The results of such evaluation indicate that (i) histone (H2A + H2B) and total histones yield reconstituted complexes whose physical properties most closely resemble those of isolated chromatin and their final complexes were relatively insensitive to the methods used in reconstitution, (ii) the physical properties of chromatin could be partially regenerated by complexes of DNA with histone (H2A + H2B + H3 + H4), H2A, or H2B, (iii) during reconstitution interaction occurs between histone H2A and H2B, between (H2A + H2B) and (H3 + H4), or between H1 and (H2A + H2B + H3 + H4), and (iv) the binding properties of histones to DNA depend not upon the DNA used in reconstitution but upon the histones and their combinations. Evaluation of reconstituted histone·DNA complexes based upon thermal denaturation and CD measurements is compared with those obtained by x-ray diffraction, nuclease digestion, and electron microscopy.

Investigation of chromosomal components, their interaction and their structure in chromatin, has utilized primarily two methods: (a) fragmentation of isolated chromatin into smaller units by shearing or nuclease digestion, followed by partial removal of histones using salt, acid or other means; (b) full dissociation and/or fractionation of chromatin components followed either by direct reconstitution from dissociated chromatin or by reconstitution using DNA and one or several isolated species of chromosomal components. To test the fidelity of reconstitution certain properties of native chromatin must be well characterized so that they can be used as objective criteria to measure the extent to which these properties can be regenerated in reconstituted complexes. Such properties can be transcriptional products (Bekhor et al., 1969; Huang and Huang, 1969; Gilmour and Paul, 1969; Stein et al., 1975; Biessman et al., 1976), morphology under the electron microscope (Oudet et al., 1975; Woodcock, 1977), higher order structure of the DNA as examined by x-ray diffraction (Richards and Pardon, 1970; Kornberg and Thomas, 1974; Boseley et al., 1976), protection of the DNA against nuclease digestion (Axel et al., 1974; Weintraub et al., 1975; Camerini-Otero et al., 1976; Sollner-Webb et al., 1976), direct binding of histones to DNA measured by thermal denaturation profiles (Li, 1977, and references therein), and conformation of DNA and proteins measured by circular dichroism (CD) (Fasman, 1977, and references therein). Each measurement reflects a certain aspect of chromatin structure; it can be related to other properties or totally unrelated. Consequently, each measurement of reconstituted chromatin or histone·DNA complexes has its own merit and should be considered in evaluating reconstitution. In addition, some histone·DNA complexes, whose physical properties may not overtly mimic those of isolated chromatin, may still reveal general aspects of the histone-DNA interaction.

In native chromatin, the binding of histones to DNA induces two phases of DNA melting at higher temperatures (Li and Bonner, 1971; Ansevin et al., 1971; Li et al., 1973; Subirana, 1973; Tsai et al., 1975). These phases were interpreted to be a result of the binding to DNA of two different regions, the less basic and the more basic halves of histone molecules (Li and Bonner, 1971). Thermal denaturation results also indicate that there are seven amino acids bound per base pair in histone-bound regions of chromatin (Li et al., 1973). Characteristic contributions to the apparent CD spectrum are made by both the DNA and proteins of chromatin (Shih and Fasman, 1970; Simpson and Sober, 1970; Hensen and Walker, 1970; Permogorov et al., 1970). Such characteristic properties, measured by thermal denaturation and CD, have been used in this report to examine reconstituted histone·DNA complexes prepared by several methods of reconstitution using DNA of different guanine + cytosine (G + C) content together with histones either individually in purified form or in various combinations. The results indicate that (a) both thermal denaturation and

† From the Department of Chemistry, Brooklyn College of the City University of New York, Brooklyn, New York 11210, and the Division of Cell and Molecular Biology, State University of New York at Buffalo, Buffalo, New York 14260. *Received May 27, 1977.* Supported by National Science Foundation Grant PCM 76-03268. H. J. L. is a recipient of a Career Development Award GM 00262 from the National Institutes of Health. Taken in part from a Ph.D. dissertation by I. M. Leffak, City University of New York, 1976.

§ Address correspondence to: Division of Cell and Molecular Biology, State University of New York at Buffalo, Buffalo, New York 14260.

‡ Present address: Department of Biochemical Sciences, Princeton University, Princeton, New Jersey 08540.

CD properties of chromatin could be largely regenerated either by complexing with a mixture of histones H2A and H2B or by using total calf thymus histones; (b) they could be partially regenerated by the use of H1-depleted histones, histone H2A or H2B; (c) the presence of urea in reconstitution does not always yield better histone-DNA complexes than in its absence; and (d) the characteristic properties of such complexes are not sensitive to the G + C content of the DNA used in reconstitution.

Materials and Methods

Calf thymus DNA was purchased from Sigma Chemical Co. and was purified by phenol extraction. *Micrococcus luteus* DNA was purchased from Miles Laboratories and *Clostridium perfringens* DNA from Worthington Biochemical Corp. These two DNAs were used without further purification. A molar absorption coefficient at 260 nm of 6500 M^{-1} cm^{-1} was used for calf thymus DNA, 7400 for *Cl. perfringens* DNA, and 7000 for *M. luteus* DNA (Felsenfeld and Hirschman, 1965).

Calf thymus chromatin was isolated according to the method of Shih and Bonner (1969). Total histones were isolated from purified chromatin by treatment with 0.4 N H_2SO_4. Dissociated histones were precipitated by absolute ethanol and stored at − 20 °C for 24 h. The pellets (histones) were washed with cold ethanol and dried under vacuum. Acrylamide gel electrophoresis (Panyim and Chalkley, 1969) showed the presence of all histone fractions (H1, H2A, H2B, H3, and H4).

Histone (H2A + H2B + H3 + H4) was prepared by dissolving the total histones in 1 mM HCl, followed by precipitation in 5% perchloric acid (PCA)[1] for 15 min (Oliver et al., 1972). The pellet was washed with 5% PCA, dissolved in 1 mM HCl dialyzed against 0.1 N HCl or H_2SO_4, and then precipitated by ethanol. This fraction was histone (H2A + H2B + H3 + H4) or H1-depleted histone. Both extracted histone H1 in the supernatant fraction and other histones in the pellet fraction were confirmed by gel electrophoresis.

Histone (H2A + H2B) fraction was purified from total histones or H1-depleted histones on Amberlite CG-50 eluted by guanidinium hydrochloride (GdmCl) (Bonner et al., 1968). The histone (H2A + H2B) fraction isolated by this method was frequently contaminated with small amounts of histone H3. The latter was removed by oxidizing the above fraction in 6 M GdmCl, 0.3 M Tris, pH 8.3, at room temperature for 24 h (Sanders and McCarty, 1972) followed by Bio-Gel P-60 column chromatography (1.6 × 200 cm) eluted with 0.01 N HCl. Histone (H2A + H2B) was found to be electrophoretically pure.

Histone H2B was purified from partially purified histone (H2A + H2B) using the procedure of Oliver et al. (1972) followed by oxidation and Bio-Gel P-100 gel filtration to remove the residual histone H3 contamination. The histone H2B obtained was electrophoretically pure. When cleaved completely by cyanogen bromide (CNBr, Eastman Chemicals) it gave only two bands with electrophoretic mobilities equivalent to those previously reported for histone H2B half-molecules (Li and Bonner, 1971).

Histone H2A was prepared by adding solid CNBr to H1-depleted histones (20 mg/mL in 0.1 N HCl) to a final CNBr concentration in excess of 25 mg/mL. The mixture was stirred at room temperature in a sealed vial for over 24 h, lyophilized, and redissolved in 0.2 N HCl and fractionated in Bio-Gel P-60 column (1.6 × 300 cm). Such fractionation was based upon the fact that histone H2A is the only histone without methionine and remains intact after CNBr treatment. The histone H2A obtained was electrophoretically pure; the purified histone H2A band migrated slightly faster than the H2A band in total histones. Despite this difference in mobility, the amino acid composition of this histone H2A was found to agree with that obtained from the reported sequence of this histone (Yeoman et al., 1972; Sautiere et al., 1974; Hnilica, 1972).

The following methods of complex formation between histone and DNA at 4 °C were used: (a) continuous NaCl gradient dialysis (2.0 to about 0.1 M) in 5 M urea, followed by a continuous urea gradient (Li and Bonner, 1971), which is a modification of the stepwise gradient dialysis first used by Bekhor et al. (1969) and Huang and Huang (1969); (b) continuous NaCl gradient dialysis (2.0 to about 0.1 M) without urea, a modification of the stepwise gradient dialysis first used by Huang et al. (1964) (in both cases, the final complexes were dialyzed to 0.25 mM EDTA, pH 8.0 (EDTA buffer)); (c) direct mixing of histone to DNA, both in EDTA buffer; (d) direct mixing of histone and DNA, both in 0.15 M NaCl, 0.25 mM EDTA, pH 8.0, followed by dialysis to EDTA buffer; (e) direct mixing of histone and DNA, both in 0.01 M sodium phosphate, pH 7.0, followed by dialysis first against 0.01 M NaCl, 0.01 M Tris, pH 8.0, and then against EDTA buffer (Yu et al., 1976a). A histone solution was first prepared in water and its concentration determined by using a molar extinction coefficient of 470 M^{-1} cm^{-1} at 230 nm (or A_{230} = 4.25 for 1 mg/mL of histone solution) (Ohlenbusch et al., 1967). It was then diluted with buffer solution to a concentration appropriate for complex formation. The initial input ratio of histone to DNA, r, in amino acids/nucleotide, was recorded for each complex.

Thermal denaturation of the complexes was made on a Gilford spectrophotometer Model 2400-S. Percent increase in absorbance at 260 nm is reported as hyperchromicity (h), its first derivative with respect to temperature as dh/dT.

Circular dichroism (CD) measurements were made 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 molar extinction coefficients of the left- and the right-handed circularly polarized light. The unit for $\Delta\epsilon$ is M^{-1} cm^{-1}, where M represents moles/liter in nucleotide for DNA and complexes, and in amino acids/liter for histones. DNA concentration in each complex was determined by absorption after correcting the light-scattering contribution to the absorbance at 260 nm according to the method of Leach and Scherage (1960). Occasionally hydrolysis of the complexes in 0.5 N $HClO_4$ followed by absorbance measurements (Adler et al., 1975b) was used and was found to give results consistent with those obtained by the former method.

Results

Thermal Denaturation Profiles of Reconstituted Histone·DNA Complexes Prepared by NaCl Gradient Dialysis in Urea. Thermal denaturation profiles of histone·DNA complexes prepared by NaCl gradient dialysis in urea are shown in Figure 1. The binding of total histones to DNA caused a decrease in the melting band of free DNA at 47 °C with a concomitant increase of two major melting bands at 74 and 83 °C with a shoulder beyond 90 °C (Figure 1a). The 90 °C shoulder was more pronounced than that found in chromatin. Otherwise, the melting profiles of these complexes were similar to that of calf thymus chromatin also shown in Figure 1a, i.e., with two melting bands at 74 and 82 °C corresponding to histone-bound DNA (Li et al., 1973).

[1] Abbreviations used: PCA, perchloric acid; GdmCl, guanidinium hydrochloride.

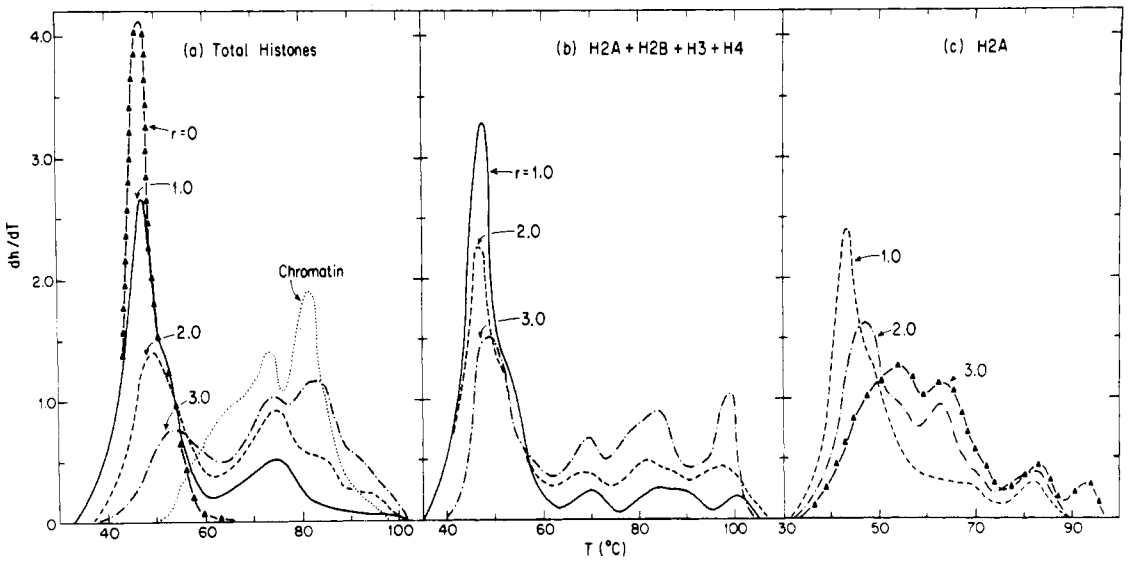

FIGURE 1: Derivative melting profiles of histone-calf thymus DNA complexes prepared by NaCl gradient dialysis in urea. Complexes formed between calf thymus DNA and (a) total histones, (b) histone (H2A + H2B + H3 + H4), or (c) histone H2A. Input ratio of histone to DNA in each complex, r, in amino acids/nucleotide is included in the figure. Also included in a is calf thymus chromatin (Li et al., 1973).

When histone H1 was excluded from the total histones, i.e., histone (H2A + H2B + H3 + H4), the melting profiles of the reconstituted complexes showed three induced melting bands at higher temperatures, 68–70 °C, 78–84 °C, and 98–100 °C (Figure 1b). The first two bands are close to the two melting bands (74 and 82 °C) of histone-bound DNA in chromatin (Figure 1a). However, the last melting band at 98–100 °C has not been observed in native chromatin. This melting band could result partly from an increase in light scattering at this temperature because the shoulder or the melting band above 90 °C became more pronounced for complexes with higher r values ($r = 3$, for example) which, in general, showed greater A_{320}/A_{260} ratio than those with lower r values. It is also noted that those reconstituted complexes with melting curves and CD spectra closer to native chromatin, e.g, (H2A + H2B)·DNA and total histones·DNA complexes, showed much smaller light scattering than those complexes, e.g., H2A·DNA or (H2A + H2B + H3 + H4)·DNA, whose melting curves and CD spectra were sufficiently different from those of native chromatin.

If the results of Figures 1a and 1b are compared with one another, the presence of histone H1 in the reconstituted mixtures seems to reduce the melting band at 98–100 °C (Figure 1b) into a small shoulder (Figure 1a). These results clearly indicate the influence of histone H1 on the binding of other histones to the DNA.

Melting profiles of native and partially dehistonized chromatins have been used to calculate the number of amino acids per nucleotide (β value) in histone-bound regions of chromatin and, in turn, the average length of DNA bound by each individual histone molecule (Li, 1973). Therefore, quantitative data obtained from thermal denaturation of histone·DNA complexes would be a good criterion to test the quality of reconstitution.

The equation used for the above calculations is

$$r = \beta\left(1 - \frac{A_{T_m}}{A_T}\right) \qquad (1)$$

where r is the input ratio in amino acids/nucleotide, A_T the total melting area, and A_{T_m} the area under the free DNA melting band. Because of the shift of the free DNA melting band, especially for complexes at higher r values, the area A_{T_m} was measured as the residual area not included in the melting bands of histone-bound DNA (melting bands with temperatures higher than that of free DNA). The slope of linear plot of eq 1 yields β, the number of amino acids per nucleotide in histone-bound regions. When total histone·DNA complexes were prepared by NaCl gradient dialysis with urea, the calculated β value was 3.5–3.7 amino acids/nucleotide or 7.0–7.4 amino acids/base pair regardless of whether the DNA was from calf thymus (42% G + C), *Cl. perfringens* (31% G + C), or *M. luteus* (70% G + C). The above value of 7.0–7.4 amino acids/base pair agrees with the value of 6.4–7.4 aa/bp found in chromatin (Li et al., 1973) and suggests that we may be observing some histone–DNA which resembles the in vivo interactions in chromatin.

Previously, it was demonstrated that the thermal denaturation profiles of complexes prepared by NaCl gradient dialysis in urea using histone (H2A + H2B) and calf thymus DNA resulted in two melting bands at 66 °C and 80 °C which were similar to those observed in chromatin (Li and Bonner, 1971). When purified histone H2B alone was used in the reconstitution, two melting bands were also obtained, one at 65–70 °C and the other at 88–90 °C (Leffak et al., 1974) which still resembled those of chromatin but not as closely as when histone (H2A + H2B) or the total histones were used in reconstitution. A logical extension of this investigation is to examine the melting properties of the complexes using histone H2A alone. The results are shown in Figure 1c. The melting profiles show a major melting band at 63–65 °C and a minor one at 83 °C. An additional band at 93 °C was observed in the complex with an input ratio equal to 3.0 amino acids/nucleotide. The first two bands are qualitatively similar to those found in reconstituted complexes formed with histone (H2A + H2B) (Li and Bonner, 1971) and with total histones or in native chromatin (Figure 1a). According to the original interpretation of these two melting bands, these results suggest that, using NaCl gradient dialysis in urea, both the more basic and the less basic regions of histone H2A or H2B can bind well enough to render thermal stabilization of the DNA in a manner similar to that observed in chromatin.

Thermal Denaturation Profiles of Reconstituted Histone·DNA Complexes Prepared by NaCl Gradient Dialysis Without Urea. The melting profiles of various histone·DNA

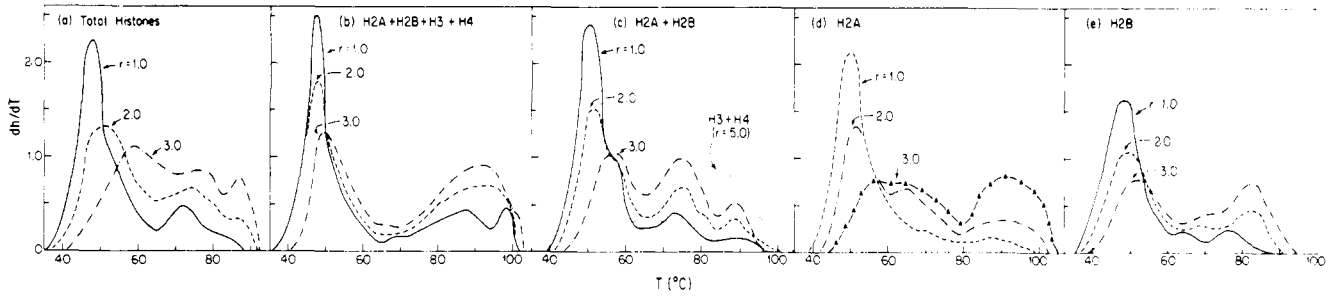

FIGURE 2: Derivative melting profiles of histone-calf thymus DNA complexes prepared by NaCl gradient dialysis without urea. Complexes formed between calf thymus DNA and (a) total histones, (b) histone (H2A + H2B + H3 + H4), or H1-depleted histones, (c) histone (H2A + H2B), (d) histone H2A or (e) histone H2B.

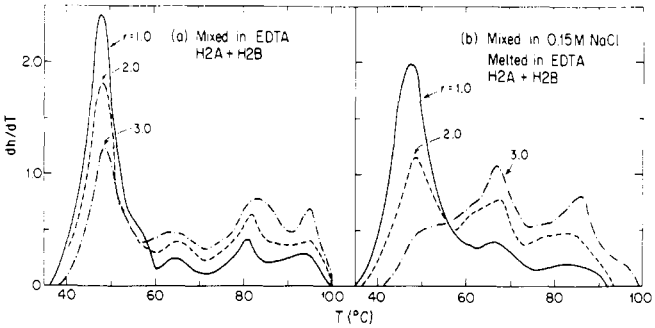

FIGURE 3: Derivative melting profiles of calf thymus DNA complexed with histone (H2A + H2B) prepared by (a) direct mixing in EDTA buffer, or (b) direct mixing in 0.15 M NaCl, but dialyzed to EDTA buffer prior to denaturation.

complexes reconstituted by NaCl gradient dialysis without urea are shown in Figure 2.

When total histones were complexed to DNA, two melting bands at 72–77 and 85–87 °C were induced, which were close to the two bands at 74 and 82 °C observed in native chromatin (Figure 1a). The β value obtained from the linear plot of eq 1 was 3.2 amino acids/nucleotide which was also close to that obtained from chromatin. Therefore, the presence of urea in the reconstitution medium does not seem to have a big effect on thermal denaturation properties of histone·DNA complexes when the total histones are used.

Removal of histone H1 from the total histones has a profound effect on the melting properties of "minus-urea" reconstituted complexes (Figure 2b). There are two higher melting bands at 87 and 98 °C for the complex with $r = 1.0$ and only one broad band near 92 °C for the complexes with $r = 2.0$ and 3.0 which differ from three higher melting bands at 68–70, 78–84, and 98–100 °C when similar complexes are prepared by reconstitution in urea (Figure 1b). Perhaps the most noticeable difference is a lack of the melting band at 68–70 °C in "minus urea" reconstituted complexes. It seems to be possible that the omission of urea from the reconstituted media has prevented the less basic regions of histones (H2A + H2B + H3 + H4) from proper binding to DNA. In other words, urea in reconstitution media seems to keep the less basic regions of these histones from histone–histone interaction and facilitates their binding to DNA.

When the slightly lysine-rich histone pair (H2A + H2B) was used in minus-urea reconstitution, the complexes exhibited two induced melting bands at 73–75 °C and 89–90 °C (Figure 2c) and a β value of 4.1 amino acids/nucleotide which was not very different from the value of 3.5 in chromatin. These melting profiles are qualitatively similar to those of histone (H2A + H2B)·DNA complexes prepared by "plus-urea" reconstitution except that the temperatures of both bands are 7–10 °C higher than those of the latter complexes (Li and Bonner, 1971). The presence of two melting bands in histones (H2A + H2B)·DNA complexes no matter whether urea is present in reconstituted media or not suggests that the absence of urea does not severely prohibit both the more basic and the less basic regions of histones from binding to DNA. Apparently the absence of urea in reconstitution media has less effect on histones (H2A + H2B)·DNA complexes than on histones (H2A + H2B + H3 + H4)·DNA complexes (Figures 1b and 2b). For comparison, one melting profile of histones (H3 + H4)·DNA complex prepared by NaCl gradient dialysis without urea is included in Figure 2c which differs from those of (H2A + H2B)·DNA complexes.

When histone H2A was complexed with DNA using "minus urea" reconstitution, one major melting band near 90 °C and a minor one near 65–70 °C were observed (Figure 2d). Comparison of Figure 2d with Figure 1c reveals that, if H2A is allowed to bind DNA without urea, it stabilized the latter to a temperature higher than that when annealed in the presence of urea.

As with histone H2A, the binding of H2B in the absence of urea still induces two high temperature transitions in DNA, a major one at 78–83 °C and a minor one at 64–70 °C. Apparently some of the thermal denaturation properties of native chromatin can be regenerated in complexes of DNA with histone H2A or H2B.

Thermal Denaturation Profiles of Histone·DNA Complexes Prepared by Other Methods or Using DNAs of Varied G + C Content. Although NaCl gradient dialysis with or without urea has been used by a number of laboratories working with reconstituted chromatin and histone·DNA or polypeptide·DNA complexes, direct mixing and other methods of complex formation have also been employed. In order to understand the dependence of the binding of histones to DNA on the conditions of complex formation, the slightly lysine-rich histone pair (H2A + H2B) was complexed with DNA using three other methods, i.e., direct mixing in 0.25 mM EDTA, pH 8.0, and direct mixing in 0.15 M NaCl or in 0.01 M sodium phosphate, pH 7.0, each followed by dialysis to 0.25 mM EDTA buffer, pH 8.0.

Figure 3a shows the derivative melting profiles of histone (H2A + H2B)·DNA complexes formed by direct mixing in 0.25 mM EDTA. These complexes exhibit three melting bands, at 65 °C, 82–84 °C, and 95 °C in addition to the original band of free DNA at about 48 °C. The 65 °C and 82–84 °C bands are qualitatively similar to those observed in chromatin (Figure 1a). The highest melting band at 95 °C, though occasionally observed in some reconstituted histone·DNA complexes (Figures 1 and 2), has not been observed in native chromatin. The highest melting band at 95 °C was diminished and only

the two chromatin melting bands were observed when histone (H2A + H2B)·DNA complexes were formed by direct mixing in 0.15 M NaCl followed by dialysis to 0.25 mM EDTA (Figure 3b). In addition, the extent of DNA binding by the less basic halves of the histones, as measured by the melting band around 67 °C, increases in the 0.15 M NaCl complexes as compared with the EDTA complexes. Presumably the two halves of histone H2A as well as H2B in the mixture can bind DNA in a manner more closely resembling that in native chromatin when they are added to DNA in 0.15 M NaCl than when they are added to DNA in 0.25 mM EDTA. This is particularly interesting because 0.15 M NaCl is close to the physiological ionic strength in the nuclei.

If histone (H2A + H2B) is added to DNA in 0.01 M sodium phosphate, pH 7.0, and then dialyzed to EDTA buffer, melting profiles similar to those of Figure 3b were observed, i.e., with two well-resolved melting bands at 65–67 °C and 82–86 °C but no melting band above 90 °C (data not shown).

Both the nuclease digestion pattern of reconstituted chromatin (Axel et al., 1974) and the melting pattern of histone H5·DNA complexes (Hwan et al., 1975) suggest that specific types of histone binding to DNA could be regenerated using DNA of varied sequences. This conclusion was tested by measuring both melting and CD properties of histone·DNA complex by varying (a) DNA, (b) histone, and (c) method of complex formation.

When total histones were complexed with *M. luteus* (70% G + C) or *Cl. perfringens* DNA (31% G + C) using NaCl-gradient dialysis in 5 M urea, melting patterns similar to that of chromatin or of reconstituted complexes using calf thymus DNA (Figure 1a) were observed (Figures 4a and 4b). The two higher melting bands were found to be 89–92 °C and 104–105 °C in the complexes of *M. luteus* DNA, and 70 and 85 °C in the complexes of *Cl. perfringens* DNA. These differences can be explained as a result of different G + C content in histone-bound regions in these two types of complexes, because, when free, *M. luteus* DNA melted at 63 °C while *Cl. perfringens* DNA melted at 39 °C with a difference of 24 °C.

Figures 4c and 4d show results of complexes formed between total histone and *M. luteus* or *Cl. perfringens* DNA prepared by NaCl gradient dialysis without urea. Aside from the shift of melting temperature, the melting patterns are similar to each other and also similar to that observed in calf thymus DNA complexes made by the same procedure (Figure 2a). Qualitatively they are also similar to those of Figures 4a and 4b when NaCl gradient dialysis in 5 M urea was used.

The above results imply that certain specific types of binding of total histones to DNA are insensitive to G + C content of DNA. Similar conclusions have been reached when histone H2A + H2B + H3 + H4, histone H2A + H2B, histone H2A or H2B alone was used in complex formation with these DNAs (Leffak, 1976).

Circular Dichroism Spectra of Reconstituted Histone·DNA Complexes Prepared by NaCl Gradient Dialysis in Urea. The circular dichroism (CD) spectra of histone·DNA complexes prepared by NaCl gradient dialysis in 5 M urea are shown in Figure 5. For those complexes of total histones, the binding of histones to DNA resulted in a large decrease in apparent CD below 240 nm, especially near 220 nm, and a substantial depression of the positive CD near 275 nm. Also included in Figure 5a is the CD spectrum of chromatin. The chromatin CD can be compared with that of the reconstituted complex with $r = 3.0$ which has a histone/DNA ratio close to that observed in native chromatin. It is noted that the negative ellipticity near 220 nm of the reconstituted complex (which reflects the degree of protein secondary structure) is about 60% that of chromatin.

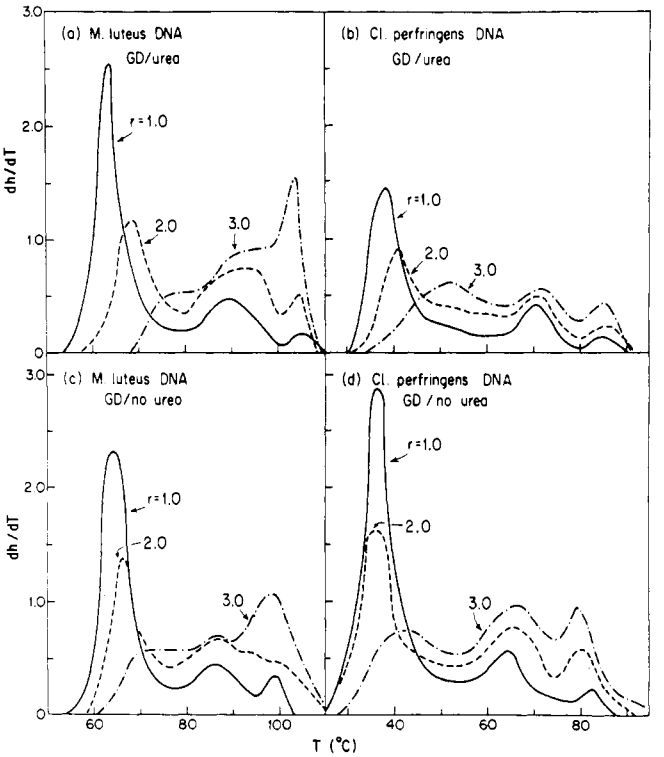

FIGURE 4: Derivative melting profiles of other DNA complexes with total histones. (a) Complexes with *M. luteus* DNA or (b) *Cl. perfringens* DNA prepared by NaCl gradient dialysis in urea (GD/urea); (c) complexes with *M. luteus* DNA or (d) *Cl. perfringens* DNA prepared by NaCl gradient dialysis without urea (GD/no urea).

Presumably the ordered secondary structure of the histones in native chromatin was not fully regenerated in the reconstituted complexes. Furthermore, the conformational change in the DNA caused by the binding of histones (as measured by the depression of the positive CD near 275 nm) is also smaller than that found in chromatin. Thus the reconstitution is not perfect as judged by the extent of regeneration of the characteristic conformations of histones and DNA in chromatin, although thermal denaturation properties of the reconstituted complexes are close to those of chromatin (Figure 1a and the above β values)

If histone H1 is omitted from the histone fraction, the apparent CD spectra of the reconstituted complexes (Figure 5b) are quite different from those in which H1 is included (Figure 5a). The amplitude of the negative ellipticity near 220 nm is reduced roughly by half when histone H1 is not present. The reduction in the positive CD band of DNA, however, was not significantly affected (Figures 5a and 5b). The smaller amount of ordered secondary structure of these histones in the reconstituted complexes (Figure 5b) could be related to the appearance of the melting band at 100 °C (Figure 1b) which is not observed in chromatin.

When only the slightly lysine-rich histone pair (H2A + H2B) was used for the reconstitution, the CD spectra of the complexes closely resembled that of native chromatin (compare the spectrum of the complex with $r = 3.0$ in Figure 5c with that of chromatin in Figure 5a). The most significant similarity between these two spectra is found in the protein region around 220 nm. This suggests that the secondary structure of the complexed histone (H2A + H2B) is close to the composite secondary structure of the histones in chromatin. However, the conformational effect on the DNA induced by the binding of histone (H2A + H2B) as judged by the positive CD band near 275 nm is smaller than that found in chromatin. For compar-

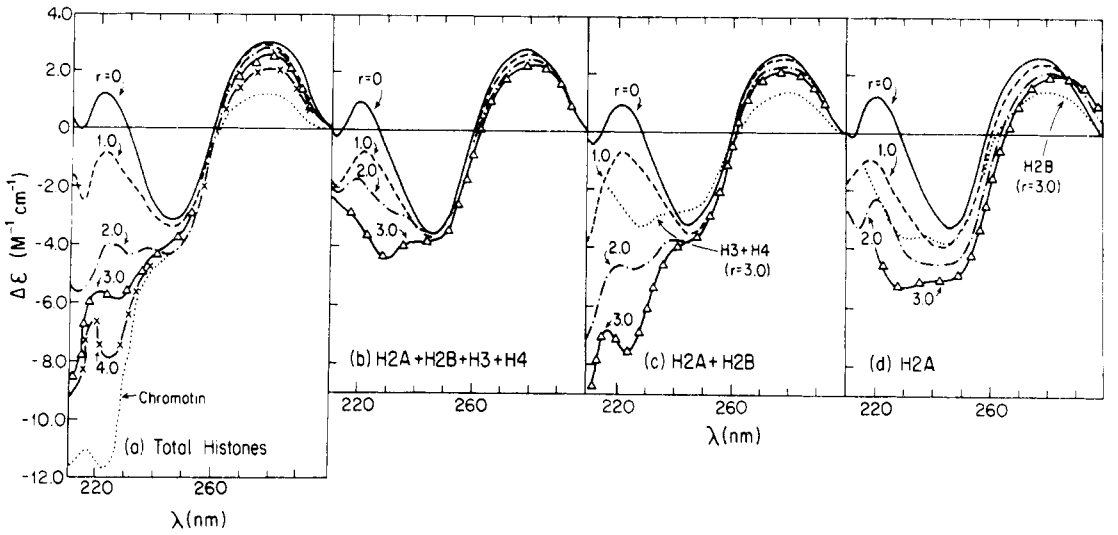

FIGURE 5: Circular dichroism spectra of histone-calf thymus DNA complexes prepared by NaCl gradient dialysis in urea. Complexes formed between calf thymus DNA and (a) total histones, (b) histone (H2A + H2B + H3 + H4), (c) histone (H2A + H2B), or (d) histone H2A. Also included are calf thymus chromatin (Li, 1975) in a, the complex with histone (H3 + H4) with r = 3.0 (Yu et al., 1976b) in c.

ison, the CD spectrum of the reconstituted histone (H3 + H4)·DNA complex with r = 3.0 (Yu et al., 1976b) is included. It is seen that arginine-rich histones in the complex possess less ordered secondary structure but have a greater effect on the DNA conformation than do the slightly lysine-rich histones.

When purified histone H2A alone is complexed with DNA, the CD spectra of the complexes (Figure 5d) are quite different from other complexes using total histones, histone (H2A + H2B + H3 + H4), or the slightly lysine-rich histone pair (H2A + H2B) (Figures 5a-c), especially in the region above 250 nm. There is a substantial red-shift in both the positive peak and the crossover point. The red-shift and the reduction in the amplitude of the positive band near 275 nm, as well as the negative CD near 220 nm in Figure 5d differ from a blue-shift and an increase of the amplitude of the positive CD band near 275 nm as well as a positive CD near 220 nm, observed in histone H2A·DNA complexes prepared by guanidinium hydrochloride gradient dialysis (Adler et al., 1975b). These differences could result from the different effects on histone conformation rendered by NaCl + 5 M urea and by GdmCl which, in turn, yield different types of final complexes with respect to both histone and DNA conformation. Also included in Figure 5d is the CD spectrum of a reconstituted histone H2B·DNA complex with r = 3.0 amino acids/nucleotide, which is qualitatively similar to but quantitatively different from that of histone H2A·DNA complex with the same r value.

It is noted that the CD of reconstituted histone (H2A + H2B)·DNA complex is not the sum of the individual CD spectra of the complexes of histone H2A·DNA and H2B·DNA complex (compare Figures 5c and 5d). The differences can be found in two wavelength regions, near 220 nm for histones and above 250 nm for the DNA. The greater negative CD near 220 nm in histone (H2A + H2B) than in H2B·DNA or H2A·DNA (compare the complexes with r = 3.0 in Figures 5c and 5d) implies interaction between histone H2A and H2B such that more ordered secondary structure is stabilized by this intermolecular interaction. This conclusion qualitatively agrees with an increase of α-helical structures when interaction between histone H2A and H2B was induced by phosphate in the absence of DNA (D'Anna and Isenberg, 1974a). The difference in the CD spectra above 250 nm in Figures 5c and 5d (both amplitude and red-shift) also suggests different conformational effects on DNA rendered by a complex of H2A and H2B or by noninteracting H2A and H2B.

Circular Dichroism Spectra of Reconstituted Histone·DNA Complexes Prepared by NaCl Gradient Dialysis without Urea. When urea was omitted from the media used for NaCl gradient dialysis, the CD spectra of complexes using various species of histones or their combinations (Figure 6) were qualitatively similar to their counterparts when urea was present during the reconstitution but with small quantitative differences (Figure 5). In particular, with the slightly lysine-rich histone (H2A + H2B), (Figure 6c), H2A or H2B (Figure 6d), the contribution to the negative CD near 220 nm by histones was noticeably greater for complexes prepared by NaCl gradient dialysis without urea than that with urea, indicating enhanced secondary structure in the absence of urea (Figures 6a, c, and d). The effect on the positive CD band of DNA near 275 nm, however, was smaller when urea was omitted from the reconstitution.

The above results with slightly lysine-rich histones are different from the effect of urea on the interactions of (H2A + H2B + H3 + H4) with DNA. In the latter case, the omission of urea in reconstitution medium reduced the amount of ordered secondary structures of the histones (a smaller negative CD near 220 nm) and a slightly greater effect on DNA conformation (a bigger reduction of the positive CD near 280 nm) (compare Figures 5b and 6b).

Circular Dichroism Spectra of Histone·DNA Complexes Prepared by Other Methods or Using DNA of Varied G + C Contents. Figure 7a presents the CD spectra of total histones·DNA complexes formed by direct mixing in 0.25 mM EDTA buffer, pH 8.0. Surprisingly they are similar to those observed in the complexes prepared by NaCl gradient dialysis in the presence of urea (Figure 5a) or in its absence (Figure 6a). Similar CD spectra were obtained also when total histones were complexed to DNA by the direct mixing in 0.15 M NaCl followed by dialysis to 0.25 mM EDTA. Apparently the conformation of the total histones in the complexes is stable and is not very sensitive to the solution conditions (NaCl or urea) used in complex formation.

When slightly lysine-rich histone (H2A + H2B) was added to calf thymus DNA in 0.25 mM EDTA, the CD spectra of the complexes (Figure 7b) were very similar to those observed in the complexes prepared by NaCl gradient dialysis in the

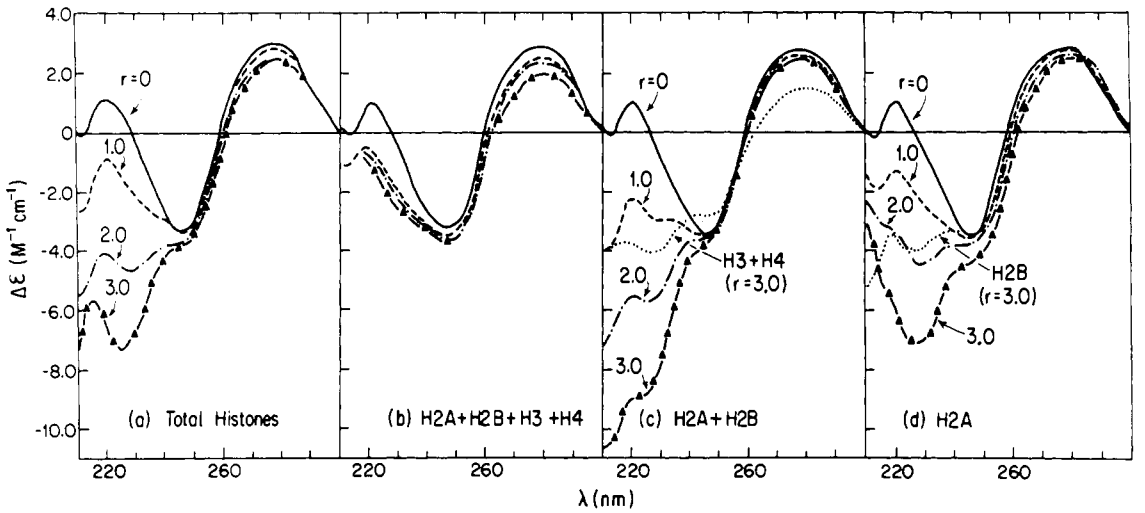

FIGURE 6: Circular dichroism spectra of histone·calf thymus DNA complexes prepared by NaCl gradient dialysis without urea. Complexes formed between calf thymus DNA and (a) total histones, (b) histone (H2A + H2B + H3 + H4), (c) histone (H2A + H2B) or (d) histone H2A. Also included are complexes with histone (H3 + H4) with r = 3.0 (Yu et al., 1976b) in c or with histone H2B with r = 3.0.

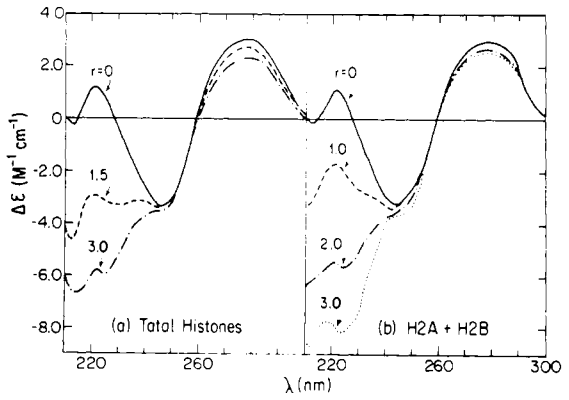

FIGURE 7: Circular dichroism spectra of calf thymus DNA complexed with (a) total histones or (b) histone (H2A + H2B) by direct mixing in EDTA buffer.

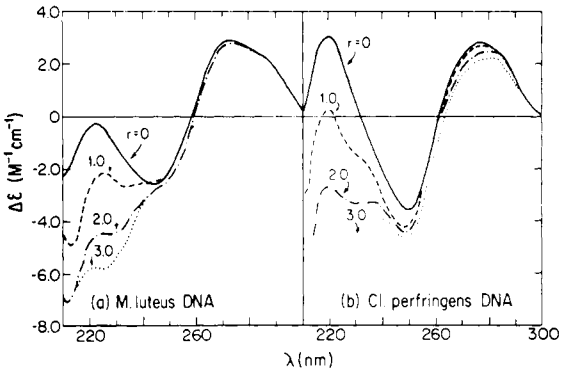

FIGURE 8: Circular dichroism spectra of other DNA complexed with total histones by NaCl gradient dialysis in urea. (a) *M. luteus* DNA or (b) *Cl. perfringens* DNA.

presence of urea (Figure 5c). Direct mixing of these histones to DNA in 0.15 M NaCl followed by dialysis to 0.25 mM EDTA buffer also yielded similar CD spectra (data not shown).

Figures 8a and 8b show CD spectra of total histones complexed with *M. luteus* DNA (70% G + C) and *Cl. perfringens* DNA (31% G + C), respectively, prepared by NaCl gradient dialysis in urea. These complexes appear different from each other. However, the differences, especially in the protein region (below 250 nm), are contributed mainly by the different CD spectra of the unbound DNAs in this wavelength region (compare the spectra of these two DNAs with r = 0). If these CD spectra are compared with those of total histones complexed with calf thymus DNA (Figure 5a), one would conclude that the protein conformation in complexes of total histones with DNAs of different G + C content is independent of the origin of DNA used in complex formation. This conclusion was found to be true for histone (H2A + H2B + H3 + H4), slightly lysine-rich histones (H2A + H2B), H2A alone or H2B alone when each histone fraction was complexed with the above three DNAs using NaCl gradient dialysis in 5.0 M urea (Leffak, 1976).

Comparison of Histone Conformation in Various Histone·DNA Complexes. For quantitative comparison of the CD at 220 nm of bound histones in the complexes ($\Delta\epsilon_b^H$), the following equation was used (Pinkston and Li, 1974):

$$\Delta\epsilon = \Delta\epsilon_f^D + r\Delta\epsilon_b^H \quad (2)$$

where $\Delta\epsilon$ is the measured CD of each complex and $\Delta\epsilon_f^D$ the CD of free DNA. The assumption made in eq 2 is that the binding of histones to DNA has negligible effect on the DNA CD near 220 nm so that the CD of free DNA ($\Delta\epsilon_f^D$) can be used for DNA in the complex. This assumption is valid when the binding of proteins to DNA causes a B→C transition in the CD spectrum of DNA. This assumption is found to be true in histone·DNA complexes examined here.

$\Delta\epsilon_b^H$ of bound histones was obtained as the slope of a linear plot of $\Delta\epsilon$ vs. r. The calculated $\Delta\epsilon_b^H$ at 220 nm was −1.8 M^{-1} cm^{-1} for histone H2A, −1.2 for H2B, −2.8 for (H2A + H2B), −1.3 for (H2A + H2B + H3 + H4), and −2.6 for total histones when the complexes were prepared in NaCl gradient dialysis in urea. When the complexes were prepared in NaCl gradient dialysis without urea, the calculated $\Delta\epsilon_b^H$ (220 nm) was −2.3 M^{-1} cm^{-1} for histone H2A, −1.9 for histone H2B, −3.3 for histone (H2A + H2B), and −2.6 for total histones; the linear relationship of eq 2 was not obeyed in the complexes of histone (H2A + H2B + H3 + H4) partly because of the small contribution of these histones to the measured CD at 220 nm (Figure 6b). When urea was not used in the reconstitution medium, there was an increase in the amplitude of the negative CD at 220 nm ($\Delta\epsilon_b^H$) for histones H2A (from −1.8 to −2.3),

H2B (from −1.2 to −1.9), and H2A + H2B (from −2.8 to −3.3), no change for the total histones (−2.6) and a decrease in histone (H2A + H2B + H3 + H4). When eq 2 was used for calculating the average $\Delta\epsilon_b^H$ (220 nm) for all histones in chromatin, the value obtained was $-4.0\ M^{-1}\ cm^{-1}$. Thus 60–80% of the secondary structure of histones in chromatin could be regenerated when slightly lysine-rich histones (H2A + H2B) or total histone were used in reconstitution. This percentage is substantially decreased when histone H2A alone, H2B alone, or (H2A + H2B + H3 + H4) was used in reconstitution.

Discussion

Reconstituted Histone·DNA Complexes Tested by Thermal Denaturation and Circular Dichroism Spectroscopy. A primary aim of reconstitution studies is to find the appropriate conditions by which a defined species or combination of histones and/or nonhistone proteins can be added to DNA in a manner simulating that in chromatin, and then determine how these components interact with one another. We attempt to discuss first the reconstitution measured by thermal denaturation and CD, and then the reconstitution measured by other methods which are related to this report.

Ansevin and Brown (1971) reported two melting bands corresponding to histone-bound DNA when histone H1, H2A, H3, or H4 was added to DNA in 3.6 M urea followed by melting in the same urea solution. Only one band was induced when histone H2B was used. Using NaCl gradient dialysis with urea, histone H2B (Leffak et al., 1974), histone H5 (Hwan et al., 1975), and histone H2A (Figure 1c) have been shown to induce two melting bands qualitatively similar to those observed in chromatin. The binding of the arginine-rich histones H3 or H4 to DNA, however, induces only one broad melting band (Yu et al., 1976a).

Fasman and co-workers (Fasman, 1977, and references therein) have reported CD spectra of reconstituted histone·DNA complexes using various species of histones, histone fragments, and modified histones as well as using several methods of reconstitution. These complexes showed B→ψ, B→C, and B→A transitions depending upon histones and method of reconstitution. Since the CD of chromatin DNA undergoes a B→C transition rather than a B→A or B→ψ transition, the discussion presented below will be limited only to those reconstituted complexes exhibiting a B→C transition.

When purified histones were used for reconstitution, a reduction in the positive CD band near 275 nm of the DNA had been observed in complexes of histone H3 or H4 (Yu et al., 1976b), H2B (Leffak et al., 1974), or H2A (Figure 5d). The red-shift of this peak and the crossover wavelength of DNA in these complexes, however, are greater than those observed with chromatin. Therefore, as far as the DNA conformation is concerned, reconstituted complexes using purified histones are still different from chromatin. Such deviation from the DNA structure in chromatin could be caused by the loss of ordered secondary structure of histones in these complexes, since the amplitude of the negative ellipticity near 220 nm in these complexes is, in general, smaller than that in chromatin (Wagner, 1970; Shih and Fasman, 1971; Adler et al., 1974, 1975a,b; Leffak et al., 1974; Yu et al., 1976b).

The quality of reconstitution can be improved substantially by the use of histone pairs, such as (H2A + H2B) or (H3 + H4), as demonstrated by both the melting (Li and Bonner, 1971; Leffak et al., 1974; Yu et al., 1976a) and CD properties (Leffak et al., 1974; Yu et al., 1976b). These results suggest initial interactions between H3 and H4 or H2A and H2B in such a way that they acquire more native and stable conformations and bind DNA in a more natural way than when only one individual species of histone is available for complexation. The results of both melting and CD results also showed that histone (H2A + H2B)·DNA complexes resembled the properties of native chromatin more closely than did those of histone (H3 + H4)·DNA complexes (Li and Bonner, 1971; Yu et al., 1976a,b; Figures 2c, 5c, and 6c).

The quality of reconstitution did not seem to be improved when a mixture of the four individual species of histone, H2A + H2B + H3 + H4, was used. The melting and CD properties of these complexes were closer to those of (H3 + H4)·DNA complexes than to those of (H2A + H2B)·DNA.

When histone H1 was included in the total histones, both the melting and CD properties of reconstituted complexes with DNA were reasonably close to those of native chromatin (Figures 1a, 2a, 5a, and 6a). Perhaps histone H1 interacts with histone (H3 + H4) or histone (H2A + H2B) [probably the former according to the report of Rubin and Moudrianakis (1975)] and modifies the interaction between histone (H3 + H4) and (H2A + H2B). Such a unit, histone (H2A + H2B) + (H3 + H4) + H1, retains about 60–70% of ordered secondary structures of chromatin proteins and facilitates the binding of both halves of each histone molecule as in chromatin.

Histone (H2A + H2B) and total histones appear to maintain a stable internal structure such that their secondary structure and binding to DNA do not change greatly when the solution conditions for making the complexes are varied.

Comparison with Other Measurements of Reconstituted Complexes. As discussed above, chromatin properties measured by melting and CD can be regenerated to a large extent in histone (H2A + H2B)·DNA but to a lesser extent in histone (H3 + H4)·DNA complexes (Yu et al., 1976a,b). In contrast, based upon x-ray (Boseley et al., 1976) and nuclease digestion criteria (Camerini-Otero et al., 1976), the reverse is true, i.e., histone (H3 + H4)·DNA complexes resemble chromatin more closely than do histone (H2A + H2B)·DNA complexes. The apparent contradiction can be resolved if the following explanations are considered:

(a) Thermal denaturation properties of a histone·DNA complex are more sensitive to the primary binding of histone to DNA than to the higher order structure of the complex (Li et al., 1973). The lower tendency toward aggregation in histone (H2A + H2B) than in (H3 + H4) might possibly allow the former to maintain its maximum amount of α-helical structure (histone CD near 220 nm) as the two halves of each molecule bind directly to the DNA (two melting bands induced by histone binding).

(b) Since histone (H2A + H2B) binds DNA more weakly than histone (H3 + H4), the former would have a higher tendency to dissociate (from the open ends of each complex fragments, for example). The higher tendency for histone (H3 + H4)·DNA to aggregate would also make this complex a poorer substrate for the nuclease as the digestion approaches the limit. Both effects may explain the observations made by nuclease digestion (Camerini-Otero et al., 1976).

(c) The higher ordered structure of DNA depends upon the distortion of its structure after histone binding. Since histone–histone interactions seem to be stronger in (H3 + H4) than in (H2A + H2B), the former might have a greater capacity to distort the secondary structure of DNA (through bending or kinking) thus leading to the appearance of higher ordered structure in DNA. In fact, judged by the depression of the positive band of DNA near 275 nm (Figures 5c and 6c), arginine-rich histone (H3 + H4) is more effective than the

slightly lysine-rich histone (H2A + H2B) in distorting the DNA conformation which is consistent with the observations made by x-ray diffraction (Boseley et al., 1976).

When histone (H2A + H2B + H3 + H4), H1-depleted histones, was used for reconstitution, the histone CD near 220 nm was much smaller than in chromatin (Figures 5b and 6b). However, the loss of the secondary structure in histones affected only the two well-defined melting bands induced by histones rather than the overall binding of histones to the DNA, because, after binding, a substantial number of base pairs were still stabilized to higher temperatures (Figures 1b and 2b). Since the higher ordered structure of DNA measured by x-ray and the presence of discrete DNA fragments at the limit of nuclease digestion were found in histone (H2A + H2B + H3 + H4)·DNA complexes, perhaps the interactions among histones and their overall binding to the DNA play more important roles in inducing higher ordered structure of DNA and in providing proper protection of DNA against nuclease digestion than do the α-helical structures of histones and the proper binding of both halves of histone molecules to the DNA.

Although the presence of histone H1 does not seem to be required for the generation of higher ordered structure (Richards and Pardon, 1970; Kornberg and Thomas, 1974; Boseley et al., 1976) for the appearance of "ν" bodies or nucleosomes (Oudet et al., 1975) or for the occurrence of nuclease-resistant fragments (Camerini-Otero et al., 1976), this histone is indeed important in stabilizing the secondary structure of other histones (Figures 5 and 6a) and in facilitating the proper binding of the two halves of each histone molecule to the DNA (Figures 1a and 2a). Perhaps histone H1 reduces the tendency toward self-aggregation in arginine-rich histones by interacting with these molecules, a phenomenon observed by Rubin and Moudrianakis (1975).

Using the appearance of dinucleosomes under the electron microscope as a criterion, Woodcock (1977) concluded that removal of urea prior to that of NaCl in reconstitution procedures yielded better histone·DNA complexes than when the order of removal of NaCl and urea was reversed. Thus urea in reconstitution media seems to disrupt irreversibly the morphology of some nucleosomes. This observation is consistent with the earlier prediction (Li, 1975) and observations (Carlson et al., 1975; Olins et al., 1977) that urea would disrupt histone–histone interaction and favor the open supercoiled structure. Such urea effect, as discussed earlier, seems to be greater on complexes of arginine-rich histones (H3 + H4) than of slightly lysine-rich histone (H2A + H2B).

The results of this report imply interactions between histone H2A and H2B, between (H2A + H2B) and (H3 + H4), as well as between H1 and (H2A + H2B + H3 + H4). Similar studies implied interactions also between histone H3 and H4 (Yu et al., 1976a,b). These implications, based upon melting and CD properties of reconstituted complexes, are consistent with other studies using chemical cross-linking techniques and electrophoresis (Kornberg and Thomas, 1974; Hyde and Walker, 1975; Hardinson et al., 1975; Martinson and McCarthy, 1975, 1976; Weintraub et al., 1975; Thomas and Kornberg, 1975) and with physical studies of histones in solution (Kelley, 1973; D'Anna and Isenberg, 1974b,c; Roark et al., 1974).

In summary, using both melting and CD properties of native chromatin as criteria, we report that (a) histone (H2A + H2B) and total histones formed subunits with stable secondary structure and bound DNA reasonably well when compared with native chromatin, (b) chromatin properties could be partially regenerated if H1-depleted histones, H2A or H2B, alone were used in reconstitution, (c) similar characteristic properties of histone·DNA complexes were obtained regardless of the G + C content or base sequence of the DNA used in complex formation and (d) histone–histone interactions occurred between histone H2A and H2B, between (H2A + H2B) and (H3 + H4), or between H1 and (H2A + H2B + H3 + H4) during reconstitution and these interactions seemed to be important in the determination of the DNA conformation in reconstituted histone·DNA complexes.

References

Adler, A. J., Fulmer, A., and Fasman, G. D. (1975a), *Biochemistry 14,* 1445.

Adler, A. J., Moran, E. C., and Fasman, G. D. (1975b), *Biochemistry 14,* 4179.

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

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

Ansevin, A. T., Hnilica, L. S., Spelsberg, T. C., and Kehn, S. L. (1971), *Biochemistry 10,* 4793.

Axel, R., Melchior, W., Jr., Sollner-Webb, B., and Felsenfeld, G. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 4101.

Bekhor, I., Kung, G. M., and Bonner, J. (1969), *J. Mol. Biol. 39,* 351.

Biessmann, H., Gjerset, R. A., Levy, W. B., and McCarthy, B. J. (1976), *Biochemistry 15,* 4356.

Bonner, J., Chalkley, R., Dahmus, M., Fambrough, D., Fujimura, F., Huang, R. C. C., Huberman, J., Jensen, R., Marushige, K., Ohlenbusch, H., Olivers, B. M., and Widholm, J. (1968), *Methods Enzymol. 12,* 3.

Boseley, P. G., Bradbury, E. M., Butler-Browne, G. S., Carpenter, B. G., and Stephens, R. M. (1976), *Eur. J. Biochem. 62,* 21.

Camerini-Otero, R. D., Sollner-Webb, G., and Felsenfeld, G. (1976), *Cell 8,* 333.

Carlson, R. D., Olins, A. L., and Olins, D. E. (1975), *Biochemistry 14,* 3122.

D'Anna, J. A., and Isenberg, I. (1974a), *Biochemistry 13,* 4987.

D'Anna, J. A., and Isenberg, I. (1974b), *Biochemistry 13,* 4992.

D'Anna, J. A., Jr., and Isenberg, I. (1974c), *Biochem. Biophys. Res. Commun. 61,* 343.

Fasman, G. D. (1977), in Chromatin and Chromosome Structure, Li, H. J., and Eckhardt, R. A., Ed., New York, N.Y., Academic Press, p 71.

Felsenfeld, G., and Hirschman, S. F. (1965), *J. Mol. Biol. 13,* 407.

Gilmour, R. S., and Paul, J. (1969), *J. Mol. Biol. 40,* 137.

Hardinson, R. C., Eichner, M. E., and Chalkley, R. (1975), *Nucleic Acids Res. 2,* 1751.

Hensen, P., and Walker, I. O. (1970), *Eur. J. Biochem. 16,* 524.

Hnilica, L. S. (1972), The Structure and Biological Functions of Histones, Cleveland, Ohio, Chemical Rubber Publishing Co.

Huang, R. C. C., Bonner, J., and Murray, J. (1964), *J. Mol. Biol. 8,* 54.

Huang, R. C. C., and Huang, P. C. (1969), *J. Mol. Biol. 39,* 365.

Hwan, J. C., Leffak, I. M., Li, H. J., Huang, P. C., and Mura, C. (1975), *Biochemistry 14,* 1390.

Hyde, J. E., and Walker, I. O. (1975), *Nucleic Acids Res. 2,* 405.

Kelley, R. I. (1973), *Biochem. Biophys. Res. Commun. 54,*

1588.
Kornberg, R. D. (1974), *Science 184,* 868.
Kornberg, R. D., and Thomas, J. (1974), *Science 184,* 865.
Leach, S. J., and Scheraga, H. A. (1960), *J. Am. Chem. Soc. 82,* 4790.
Leffak, I. M., (1976), Ph.D. Thesis, City University of New York.
Leffak, I. M., Hwan, J. C., Li, H. J., and Shih, T. Y. (1974), *Biochemistry 13,* 1116.
Li, H. J. (1973), *Biopolymers 12,* 287.
Li, H. J. (1975), *Nucleic Acids Res. 2,* 1275.
Li, H. J. (1977), Chromatin and Chromosome Structure, Li, H. J., and Eckhardt, R. A., Ed., New York, N.Y., Academic Press, p 37.
Li, H. J., and Bonner, J. (1971), *Biochemistry 10,* 1461.
Li, H. J., Chang, C., and Weiskopf, M. (1973), *Biochemistry 12,* 1763.
Martinson, H. G., and McCarthy, B. J. (1975), *Biochemistry 14,* 1073.
Martinson, H. G., and McCarthy, B. J. (1976), *Biochemistry 15,* 4126.
Ohlenbusch, H. H., Olivera, B. M., Tuan, D., and Davidson, N. (1967), *J. Mol. Biol. 25,* 229.
Olins, D. E., Bryan, P. N., Olins, A. L., Harrington, R. E., and Hill, W. E. (1977), *Biophys. J. 17,* 114a.
Oliver, D., Sommer, K. R., Panyim, S., Spiker, S., and Chalkley, R. (1972), *Biochem. J. 129,* 349.
Oudet, P., Cross-Bellard, M., and Chambon, P. (1975), *Cell 4,* 281.
Panyim, S., and Chalkley, R. (1969), *Biochemistry 8,* 3972.
Permogorov, U. I., Debavov, U. G., Sladkova, I. A., and Rebentish, B. A. (1970), *Biochim. Biophys. Acta 199,* 556.
Pinkston, M. F., and Li, H. J. (1974), *Biochemistry 13,* 5227.
Richards, B. M., and Pardon, J. F. (1970), *Exp. Cell Res. 62,* 184.
Roark, D. E., Geoghegan, T. E., and Keller, G. H. (1974), *Biochem. Biophys. Res. Commun. 59,* 542.
Rubin, R. L., and Moudrianakis, E. N. (1975), *Biochemistry 14,* 1718.
Sanders, L. A., and McCarty, K. S. (1972), *Biochemistry 11,* 4216.
Sautiere, P., Tyrou, D., Laine, B., Mizon, J., Ruffin, P., and Biserte, G. (1974), *Eur. J. Biochem. 41,* 563.
Shih, T. Y., and Bonner, J. (1969), *Biochim. Biophys. Acta 182,* 3035.
Shih, T. Y., and Fasman, G. D. (1970), *J. Mol. Biol. 52,* 125.
Shih, T. Y., and Fasman, G. D. (1971), *Biochemistry 10,* 1675.
Simpson, R. B., and Sober, H. A. (1970), *Biochemistry 9,* 3103.
Sollner-Webb, B., Camerini-Otero, R. D., and Felsenfeld, G. (1976), *Cell 9,* 179.
Stein, G. S., Mans, R. J., Gabbey, E. J., Stein, J. L., Davis, J., and Adawadkar, P. D. (1975), *Biochemistry 14,* 1859.
Subirana, J. A. (1973), *J. Mol. Biol. 74,* 363.
Thomas, J. O., and Kornberg, R. D. (1975), *Proc. Natl. Acad. Sci. U.S.A. 72,* 2626.
Tsai, Y. H., Ansevin, A. T., and Hnilica, L. S. (1975), *Biochemistry 14,* 1257.
Wagner, T. E. (1970), *Nature (London) 227,* 65.
Weintraub, H., Palter, J., and Van Lente, F. (1975), *Cell 6,* 85.
Woodcock, C. L. F. (1977), *Science 195,* 1350.
Yeoman, L. C., Olson, M. O. J., Sugano, H., Jordan, J. J., Taylor, C. W., Starbuck, W. C., and Busch, H. (1972), *J. Biol. Chem. 247,* 6018.
Yu, S. S., Li, H. J., and Shih, T. Y. (1976a), *Biochemistry 15,* 2027.
Yu, S. S., Li, H. J., and Shih, T. Y. (1976b), *Biochemistry 15,* 2034.