Conformational Effects of Organic Solvents on Histone Complexes†

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ABSTRACT: Changes in the conformations of H3–H4, H2A–H2B, and the core histone complex brought about by the addition of organic solvents have been examined by circular dichroism spectroscopy. All three complexes assume increased α helicity with increasing amounts of the organic solvent. An amount of secondary structure equal to that obtained in phosphate-buffered 2 M NaCl solution can be induced in low-salt solutions of the complexes by the addition of 40–50% ethylene glycol, 50% glycerol, or approximately 2% hexafluoro-2-propanol. H3–H4 was found to be somewhat more flexible than H2A–H2B in its response to changes in solvent polarity. Upon being heated, H3–H4 and the core histone complex both undergo irreversible $\alpha \rightarrow \beta$ transitions in 50% ethylene glycol under low-salt conditions, while H2A–H2B undergoes an essentially reversible $\alpha \rightarrow$ random-coil transition under the same conditions. These results are discussed in terms of the dynamics of the nucleosome particle.

The fundamental role of the histone class of proteins in chromosomal DNA condensation was implicated with the introduction of the nucleosome model of chromatin [for recent reviews, see Felsenfeld (1978), Isenberg (1979), Lilley & Pardon (1979), and McGhee & Felsenfeld (1980)]. According to the model, DNA is organized into compact beadlike units, with approximately 200 base pairs (bp) per unit, 145 of which are tightly coiled about a core of histone protein (Woodcock, 1973; Hewish & Burgoyne, 1973; Olins & Olins, 1974; Kornberg, 1974; Van Holde et al., 1974; Noll, 1974; Oudet et al., 1975; Ramsay-Shaw et al., 1976). This core particle contains two of each of the core histones H2A, H2B, H3, and H4 (Van Holde et al., 1974). A fifth histone, H1 (and H5 in avian erythrocytes), lies outside the core particle, closely associated with the remaining 55 or so base pairs, and has been implicated in nucleosomes spacing (Noll & Kornberg, 1977; Morris, 1976a,b; Wilhelm et al., 1977) and control of higher order structure (Renz et al., 1977; Campbell & Cotter, 1977).

The primary sequence of each of the four core histones contains two distinct domains: a region rich in cationic residues, mostly lysines and arginines, near the N terminus and a relatively neutral region of hydrophobic residues near the middle of the sequence (Van Holde & Isenberg, 1975). While elucidation of these domains (DeLange et al., 1979; Bustin et al., 1969) led to the suggestion that each has a specific function in the nucleosome architecture, the exact topology of the DNA and histones in nucleosomes and chromatin is still a subject of much research. An excellent discussion of this area can be found in McGhee & Felsenfeld (1980).

Noncovalent histone complexes containing two or more core histones have been isolated free of DNA; the more intensely studied complexes are a dimer of H2A and H2B (Kelley, 1973; D'Anna & Isenberg, 1974a; Moss et al., 1976a), a tetramer of H3 and H4 (D'Anna & Isenberg, 1974b; Roark et al., 1974; Kornberg & Thomas, 1974; Sperling & Bustin, 1975; Moss et al., 1976), and the entire core histone octamer (Thomas & Butler, 1977; Chung et al., 1978; Eickbush & Moudrianakis, 1978; Godfrey et al., 1980; Stein & Page, 1980).

The structures of the free histone complexes have been compared with the protein structure found inside the nucleosome. Similarities can be detected by infrared spectroscopy (Cotter & Lilley, 1977), laser Raman spectroscopy (Thomas et al., 1977), chemical cross-linking (Thomas & Kornberg, 1975; Thomas & Butler, 1977), and circular dichroism spectroscopy (Fulmer & Fasman, 1979), provided that the free histone complexes are maintained in a high ionic strength environment, generally that of about 2 M NaCl. Gradual lowering of the ionic strength results in progressive loss of secondary, tertiary, and quaternary structure in the free complexes (Olins et al., 1977; Bidney & Reeck, 1977; Eickbush & Moudrianakis, 1978; Ruiz-Carrillo & Jorcano, 1979; Beaudette et al., 1981). It is generally believed that the creation of a high ionic strength environment shields unfavorable electrostatic repulsions within and between the core histones, allowing for the formation of favorable secondary and higher order structure.

Differences in the structure of actively transcribing chromatin and inactive chromatin have been observed and are reviewed by McKnight et al. (1979) and Mathis et al. (1980). Transcriptionally active chromatin appears to be in a more open form since it is more readily digested by nucleases (Gottesfeld & Butler, 1977). Despite our insufficient understanding of the structure of in situ actively transcribing chromatin, it is expected that histone acetylation, phosphorylation, or methylation may have some control in the accessibility of active genes to RNA polymerase. A study by Szopa et al. (1980) suggests that phosphorylation of H3 greatly reduces its ability to associate the H4 to form the $(H3–H4)_2$ tetramer. However, phosphorylation of H3 (and H1) has also been linked to the condensation of chromatin during mitosis (Gurley et al., 1978).

To date, little information has been obtained to suggest the possibility of additional conformational stability arising from the interaction of core histones with the uncharged regions of DNA, i.e., with ribosyl groups or with the bases themselves. These regions are considerably less polar in character than the charged and highly hydrated phosphates. Perhaps of greater significance, the presence of DNA shell partially surrounding the core histones is likely to bring about exclusion of water molecules from contact with some of the protein surface. This could result in a higher propensity for intrapeptide hydrogen bonding and hence stabilization of secondary structure within these regions. Stabilization of secondary structure is also likely to result from the altered environment polarity and loss of water at the hydrophobic histone–histone and potential hi-

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stone–nonhistone contact surfaces. Conformational changes of histones could bring about a change in binding to DNA and consequently change the accessibility of the DNA to various enzymes and binding proteins. Such structural changes could be important in the function of "activity" of the nucleosome particle.

To investigate the effect of environment polarity on histone structure, we have undertaken a systematic study of the effects of organic solvent–water mixtures on the circular dichroism spectra of the histone complexes. Ethylene glycol, glycerol, and 1,1,1,3,3,3-hexafluoro-2-propanol (HFP)[1] were chosen for their relatively low polarity compared to water. Our results show that, even in the absence of high salt concentrations, significant secondary structure can be induced in the histones by reducing the polarity of the solution environment. Indeed, with the proper addition of any of the solvents tested, an amount of secondary structure equal to that obtained at high salt can be achieved. H3–H4 was found to be somewhat more flexible than H2A–H2B in its response to changes in solvent polarity. The significance of these results is discussed in terms of the dynamics of the nucleosome particle.

Materials and Methods

Isolation of Chromatin and Histone Complexes. Chicken erythrocyte nuclei were prepared by the method of Ramsay-Shaw et al. (1976). High molecular weight chromatin was solubilized by digestion with micrococcal nuclease at 37 °C and extracted twice with cold EDTA, as described by Fulmer & Fasman (1979). H2A–H2B and $(H3–H4)_2$ were purified by the hydroxylapatite method of Simon & Felsenfeld (1979) as modified by Beaudette et al. (1981). Core histone complex was isolated by the method of Butler et al. (1979) on sucrose gradients and agarose columns as described by Beaudette et al. (1981). Unless otherwise noted, all preparative procedures and subsequent handling of the histones were done at 4–5 °C. Histones were stored in 2-mL plastic Nunc tubes (InterMed, Denmark). The ratios of the histones in the various complexes were shown to be identical, based on polyacrylamide–acetic acid–urea gels [see Table I in Beaudette et al. (1981)].

Preparation of Histone–Solvent Mixtures. Histone complexes were transferred from 2 M NaCl, 10 mM sodium phosphate, 0.25 mM EDTA, and 0.1 mM DTT, pH 7.0, to 10.0 mM sodium phosphate and 0.10 mM DTT, pH 7.0, by overnight dialysis [Spectrapor 3 membrane tubing (Spectrum Medical Industries) treated as previously described (Beaudette et al., 1981)] at 4 °C. After dialysis, the pairs were filtered through Nucleopore polycarbonate filters (0.2-μm average pore diameter; the core histones were centrifuged at 12000*g* for 30 min) to remove occasional traces of aggregated material. Ultraviolet spectra obtained after filtration routinely illustrated negligible light scattering. Protein concentrations were determined by the method of Lowry et al. (1951) with purified H4 as the standard. The pairs were finally introduced into the solvent of interest by dilution into an appropriately concentrated solvent solution. After dilution, the final concentrations of sodium phosphate and DTT were 5.0 and 0.05 mM, respectively; this solvent will be referred to as phosphate–DTT buffer. All concentrations of organics are given in units of volume percent. Because of the small volumes employed, solutions were made without considering partial volume mixing effects. However, large-scale estimation of partial volume effects revealed that, at best, they resulted in a 2% error in concentration. H2A–H2B and H3–H4 were also dialyzed into 1.0 M sodium perchlorate at 5 °C for dialysis.

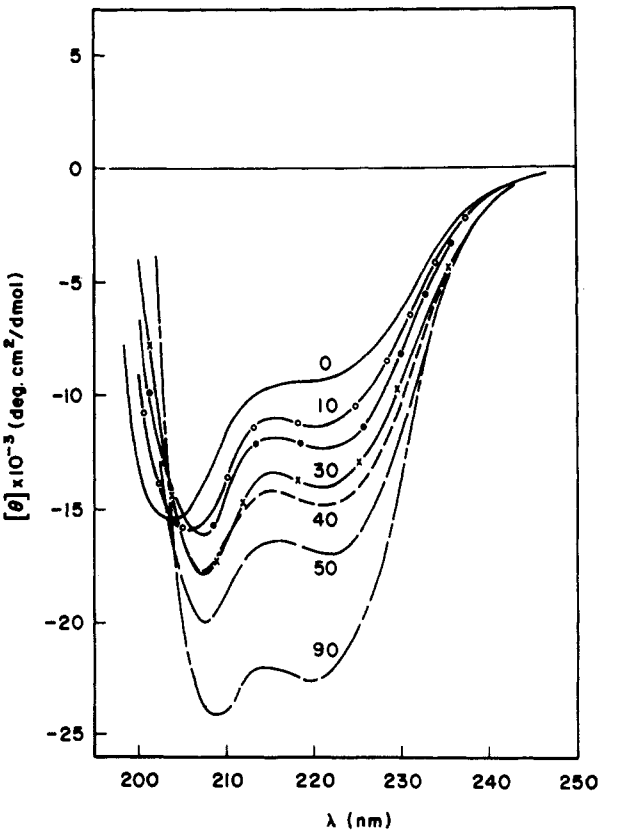

FIGURE 1: Circular dichroism spectra of the peptide region of H3–H4 at various ethylene glycol concentrations. All solutions contained 5.0 mM sodium phosphate and 0.05 mM DTT, pH 7.0 (pH measured prior to glycol addition). Spectra were recorded in a 0.1-cm jacketed cell at 5.0 °C; the histone concentration was 0.18 mg/mL. The concentration of ethylene glycol in units of volume percent was as follows: (a) 0 (—); (b) 10 (○); (c) 20 (●); (d) 30 (×); (e) 40 (---); (f) 50 (– –); (g) 90 (– – –).

Solvents. Ethylene glycol and glycerol were obtained from Fisher and were of certified ACS reagent grade. 1,1,1,3,3,3-Hexafluoro-2-propanol (99+% pure) was obtained from Eastman Chemicals. Sodium perchlorate (99+% pure) was obtained from the GFS Chemical Co. Dithiothreitol was obtained through Sigma Chemical Co. All reagents were used without further purification.

Circular Dichroism Measurements. All circular dichroism (CD) spectra were obtained with a Cary 60 recording spectropolarimeter equipped with a Model 6001 CD accessory, as previously described (Ong et al., 1976), with the original photomultiplier tube replaced with an end-on Hamamatsu tube, No. R375. All measurements were made at 5 ± 1 °C (except temperature studies) in 0.1–1.0 mm path length jacketed cells (Optical Cell Co.) at a full-scale sensitivity of 0.04°. Ellipticity, $[\theta]$, is expressed in deg cm²/dmol of amino acid residues.

Estimation of Secondary Structure. The percent of α-helical, β-sheet, and random-coil structure was calculated from the CD spectra as described by Beaudette et al. (1981). For these analyses, the α-helical and β-sheet spectra of poly(L-lysine) and the random spectrum of the histones were used as a basis set.

Results

Ethylene Glycol. The CD spectra of H3–H4, at 0.18 mg/mL, taken at 5 °C, in phosphate–DTT buffer at various

[1] Abbreviations: CD, circular dichroism; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; HFP, 1,1,1,3,3,3-hexafluoro-2-propanol; phosphate–DTT buffer, 5.0 mM sodium phosphate and 0.05 mM DTT, pH 7.0.

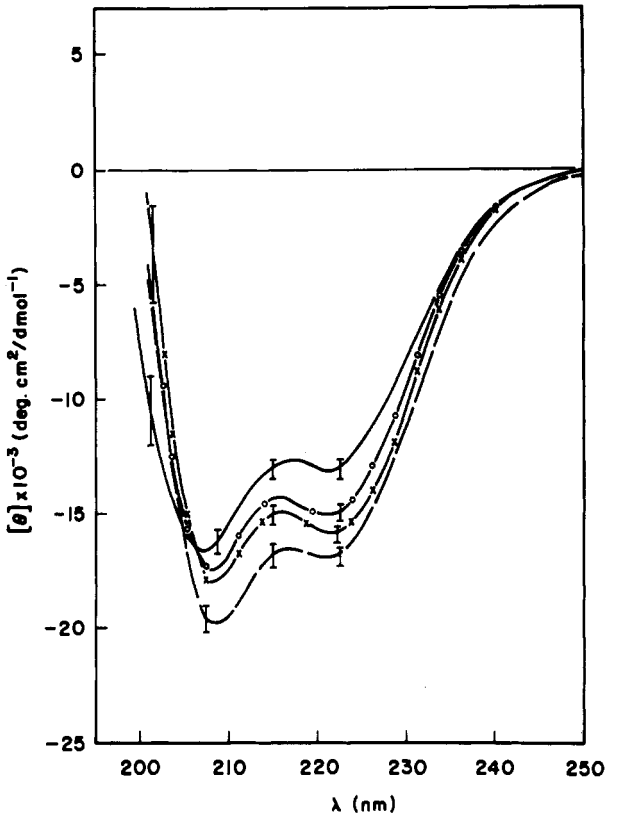

FIGURE 2: Peptide CD spectra of core histone complex at various ethylene glycol concentrations. Solution conditions were as described in the legend to Figure 1 except that the histone concentration was 0.37 mg/mL and the cell path length was 0.05 cm. The concentration of ethylene glycol in units of volume percent was as follows: (a) 0 (—); (b) 10 (O); (c) 30 (×); (d) 50 (--).

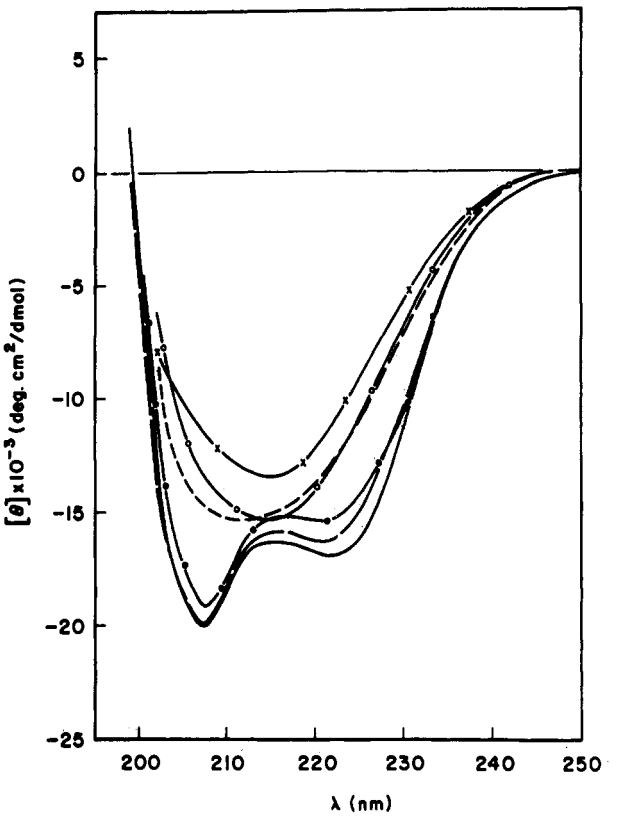

FIGURE 3: Thermal CD melt of H3–H4 in 50% (v/v) ethylene glycol, 5.0 mM sodium phosphate, and 0.05 mM DTT, pH 7.0. Spectra were recorded in a 0.1-cm jacketed cell at a histone concentration of 0.18 mg/mL. Temperature: (a) 5 °C (—); (b) 15 °C (--); (c) 20 °C (●); (d) 26.5 °C (---); (e) 65 °C (×); (f) 5 °C (O), after cooling from 65 °C.

ethylene glycol concentrations are shown in Figure 1. All studies were performed at 5 °C to eliminate aggregation, which occurred slowly at room temperature. The spectra show a strong dependence on glycol concentration. In the absence of glycol, ellipticity extrema were observed at 204 ($[\theta]_{204}$ = −15 400 deg cm²/dmol) and 220 nm ($[\theta]_{220}$ = −9400 deg cm²/dmol). Both extrema became more negative as the glycol concentration was increased. At 50% ethylene glycol, extrema were observed at 208 ($[\theta]_{208}$ = −20 000 deg cm²/dmol) and 222 nm ($[\theta]_{222}$ = −17 000 deg cm²/dmol), and at 90% ethylene glycol, the values of $[\theta]_{209}$ = −24 200 deg cm²/dmol and $[\theta]_{222}$ = −22 600 deg cm²/dmol were obtained. Computer estimation of α-helical and β-sheet structure revealed an increase in α content from 19% in the absence of ethylene glycol to 44% in 50% glycol and 61% in 90% ethylene glycol. The β-sheet content remained low (0–4% estimated) over the entire glycol concentration range studied.

H2A–H2B, at 0.24 mg/mL, exhibited similar behavior with increasing glycol concentration (data not shown); however, the changes in ellipticity were not as great as with H3–H4. In phosphate–DTT buffer without added glycol, extrema were observed at 208 ($[\theta]_{208}$ = −16 900 deg cm²/dmol) and 221 nm ($[\theta]_{221}$ = −15 000 deg cm²/dmol). At 50% glycol, negative ellipticities increased to $[\theta]_{208}$ = −19 500 deg cm²/dmol and $[\theta]_{221}$ = −17 600 deg cm²/dmol, indicating an increase in α helicity from 35% without glycol to 42% in 50% glycol. Estimates of the β-sheet structure remained low (5–15%).

The dependence of core ellipticity (0.37 mg/mL) on ethylene glycol concentration (Figure 2) could, within experimental error, be described as the weight average of the ellipticities of the pairs. Helical structure ranged from 32% to 46%, in the absence of glycol and 50% glycol, respectively. β sheet varied randomly between 0 and 9% over the same range.

The effect of heating H3–H4 in 50% ethylene glycol and phosphate–DTT buffer from 5 to 65 °C is shown in Figure 3. Loss of α structure and formation of β structure are suggested by the shift to a single extremum at 215 nm ($[\theta]_{215}$ = −13 500 deg cm²/dmol). Computer estimates show a decrease in α-helical content from 44% at 5 °C to 17% at 65 °C with a concomitant increase in β-sheet content from 0 to 20%. When the temperature was lowered back to 5 °C, the ellipticity changed only slightly, indicating that the α → β transition was irreversible. No change in the spectrum was observed over the next 5 days. The T_m for the transition with increasing temperatures was about 23 °C. H3–H4 precipitated when heated in the absence of ethylene glycol.

As H2A–H2B was heated from 5 to 63 °C in 50% ethylene glycol and phosphate–DTT buffer (data not shown), the CD spectra showed a loss of helical structure from 43% to 21% with a T_m at around 37 °C. This was paralleled by a loss of β-sheet structure from ~13% to ~3%. Hence, the transition is best described as an α → coil transition. When the temperature was lowered back down to 4.5 °C, a spectrum similar, but not quite identical, to the original low-temperature spectrum was obtained, yielding structure estimates of 39% α helix and 10% β sheet. Hence, in contrast to the α → β transition of H3–H4, the α → coil transition of H2A–H2B appears nearly reversible.

When the core protein was subjected to the same thermal analysis, in 50% ethylene glycol, the CD spectrum also became more β-like at the higher temperatures (Figure 4). At 65 °C, a single extremum was observed at 215 nm ($[\theta]_{215}$ = −10 000 deg cm²/dmol). Lowering the temperature to 5 °C did not

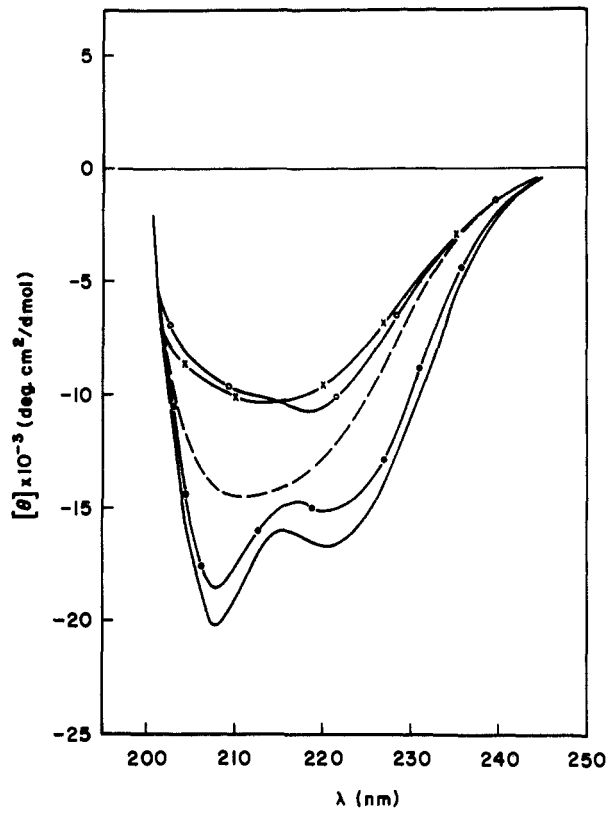

FIGURE 4: Thermal CD melt of core histone complex in 50% (v/v) ethylene glycol, 5.0 mM sodium phosphate, and 0.05 mM DTT, pH 7.0. Spectra were recorded in a 0.05-cm jacketed cell at a histone concentration of 0.40 mg/mL. Temperature: (a) 5 °C (—); (b) 25 °C (●); (c) 45 °C (---); (d) 65 °C (×); (e) 5 °C (○), after cooling from 65 °C.

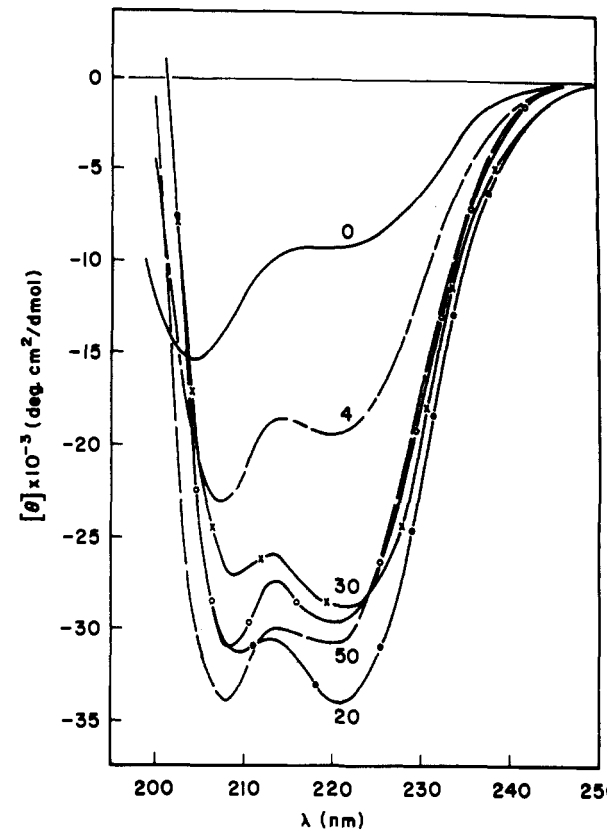

FIGURE 5: Peptide CD spectra of H3–H4 at various concentrations of hexafluoro-2-propanol. All solutions contained 5.0 mM sodium phosphate and 0.05 mM DTT, pH 7.0 (pH measured prior to addition of alcohol). Spectra were recorded in a 0.05-cm jacketed cell at 5.0 °C; the histone concentration was 0.21 mg/mL. The concentration of hexafluoro-2-propanol in units of volume percent was as follows: (a) 0 (—); (b) 4 (---); (c) 10 (○); (d) 20 (●); (e) 30 (×); (f) 50 (--).

result in a significant change in the spectrum from that obtained at 65 °C. At 65 °C, estimated α and β structure were 14% and 13%, respectively.

Hexafluoro-2-propanol. The CD spectra of H3–H4 at various concentrations of HFP are shown in Figure 5. With only 4% HFP, the negative ellipticity increased to −23 100 deg cm^2/dmol at 208 nm and −19 500 deg cm^2/dmol at 221 nm. Under these conditions, α content was estimated to be 51%, and no β content was found. When the HFP level was increased to 50%, negative ellipticity extrema increased to −34 200 deg cm^2/dmol at 207 nm and −30 800 deg cm^2/dmol at 220 nm. The increases were not uniform. As shown in Figure 5, at 20% and 30% HFP, ellipticities were more negative for the band around 220 nm than for the band around 208 nm, and ellipticities were all less negative at 30% HFP than at 20%. The origin of this behavior is not clear. At 50% HFP, α content was estimated at 85%. However, a better fit was obtained at 20% HFP, for which 99% α content was predicted. No β content was found at any level of HFP examined (from 4% to 50% HFP).

H2A–H2B exhibited similar structural sensitivity to HFP, as well as a similar reversal of band ratios at 20% HFP (data not shown). Core histone was examined only at 50% HFP. At this concentration of HFP, α content estimated for H2A–H2B and core was 97% and 86%, respectively.

Solvent Comparisons. Comparisons of the CD spectra obtained for H3–H4, H2A–H2B, and core, under various solvent conditions, including 2.0 M NaCl, 50% glycerol, 40–50% ethylene glycol, 4% HFP, and 1.0 M $NaClO_4$ (H2A–H2B only), are found in Figures 6–8. The H3–H4 complex assumed approximately the same conformation in 50% glycerol, in 40% ethylene glycol, and in 2.0 M NaCl, 10.0 mM sodium phosphate, and 0.10 mM DTT, pH 7.0 (Figure 6). The conformation is approximately 45% α helical, the remainder being random coil. In a lower ionic strength buffer (5.0 mM sodium phosphate and 0.05 mM DTT, pH 7.0) the complex has a reduced α-helical content of 19%, while in 4% HFP the α-helical content is 50%.

The α-helical content of the H2A-H2B dimer (Figure 7) increases from 35% in phosphate–DTT buffer to 45% in 50% glycerol, 50% ethylene glycol, 1.0 M $NaClO_4$, or 2.0 M NaCl–phosphate–DTT buffer. In 4% HFP, the helical content increases further to 54%.

The core histone octamer (Figure 8) assumes approximately the same conformation ($\simeq$45% α helical) in either 50% glycerol, 50% ethylene glycol, or 2.0 M NaCl. In the phosphate–DTT buffer, the core assumed a 32% α-helical structure.

In summary, the following has been shown. (a) All three histone complexes (it cannot be assumed that histone–histone interactions are retained under all conditions represented in the figures) exhibit considerable conformational flexibility; α helix and random coil are the more prominent structures present. (b) The high-salt spectra of all three complexes can be reproduced at low salt by the addition of ethylene glycol to 40–50%, glycerol to ~50%, or HFP to ~2% (estimated). At 1.0 M $NaClO_4$, H2A–H2B retains its high-salt spectrum.

Discussion

Circular dichroism spectroscopy has been used in the past to detect conformational changes in the proteins within chromatin and in isolated core histone complexes as a function of urea concentration, ionic strength, and organic solvents.

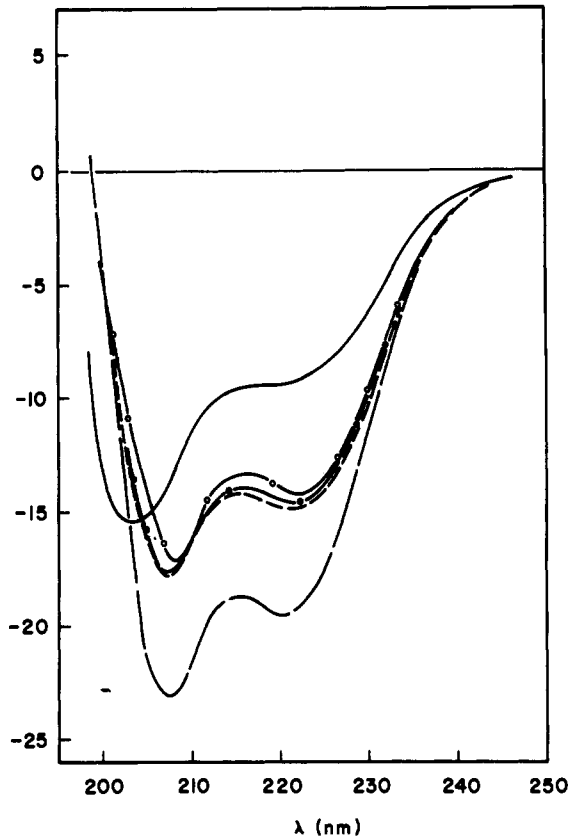

FIGURE 6: Peptide CD spectra of H3–H4 under various solvent conditions. All spectra were recorded at 5 °C with 0.05- or 0.1-cm jacketed cells at histone concentrations of 0.18–0.21 mg/mL (except the 2.0 M salt spectrum; see below). Solvent conditions: (a) 5.0 mM sodium phosphate and 0.05 mM DTT, pH 7.0 (—); (b) as in (a) with 50% (v/v) glycerol added (●); (c) as in (a) with 40% (v/v) ethylene glycol added (---); (d) as in (a) with 4% (v/v) hexafluoro-2-propanol added (--); (e) 2.0 M NaCl, 10.0 mM sodium phosphate, 0.10 mM DTT, pH 7.0, and 1.6 mg/mL histone and 0.01-cm path length (○).

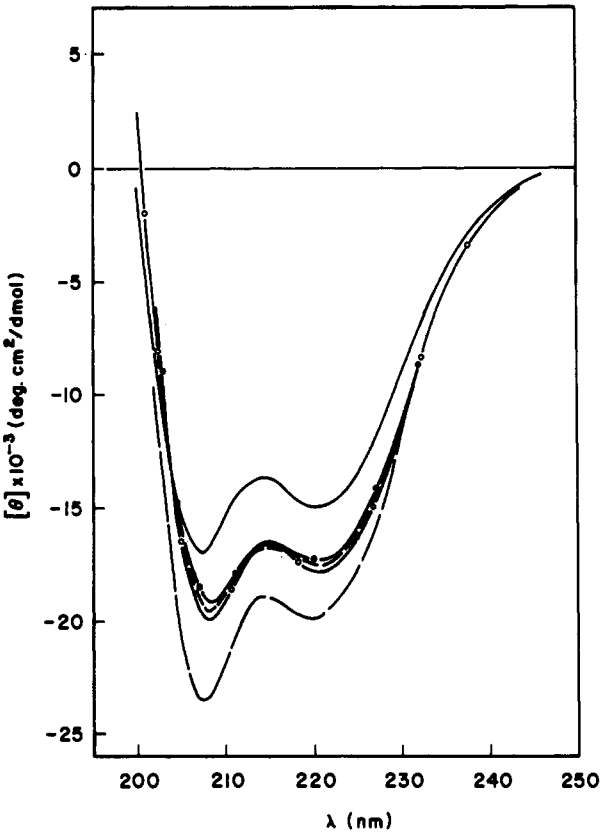

FIGURE 7: Peptide CD spectra of H2A–H2B under various solvent conditions. Spectra were recorded as in the legend to Figure 6, with histone concentrations from 0.24 to 0.38 mg/mL. Solvent conditions: (a) 5.0 mM sodium phosphate and 0.05 mM DTT, pH 7.0 (—); (b) as in (a) with 50% glyerol or 50% ethylene glycol added (●); (c) as in (a) with 4% hexafluoro-2-propanol added (--); (d) 1.0 M sodium perchlorate (---); (e) 2.0 M NaCl, 10.0 mM sodium phosphate, 0.10 mM DTT, pH 7.0, and 0.52 mg/mL histone (○).

Olins et al. (1977) observed that, at low urea concentrations (<3 M), the CD of DNA in the nucleosome changes progressively toward the B form, while histone secondary structure remains unaffected. Above 3 M urea, cooperative disruption of histone secondary structure occurs. Moss et al. (1976a,b) also found that both H2A–H2B and H3–H4 lose all secondary structure in 6 M urea. The ability of urea to disrupt inner histone secondary structure (in the absence of DNA) is decreased greatly with increasing salt concentration (Olins et al., 1977). However, at low ionic strength, histones remain associated with DNA in chromatin, even when treated with 5 M urea (Chalkley & Jensen, 1968; Chang & Li, 1974). These observations suggest that histone–histone interactions are more readily disrupted by urea than the histone secondary structure. On the other hand, high-salt concentrations generally lead to increased stability of histone secondary and quaternary structure, along with decreased histone–DNA affinity (Eickbush & Moudrianakis, 1978; Olins et al., 1977). The reversibility of histone secondary structure as a function of salt concentration has also been demonstrated (Beaudette et al., 1981).

Combining CD and fluorescence spectral data, Zama et al. (1978) found that organic solvents induce both slight and sharp transitions in the nucleosome conformation. No significant differences in the structure of nucleosomal vs. free DNA were detected although a slight increase in apparent histone α-helix content accompanied the addition of ethylene glycol up to 75%. Above 75% glycol, sharp increases in both $-[\theta]_{223}$ and $[\theta]_{281}$ appeared, suggesting increased α-helix stability in the histones and sudden disruption of DNA supercoiling. Both transitions were irreversible.

Schwartz & Fasman (1979) examined the effect of low ethylene glycol levels [1 M = 6.2% (w/w)] on the thermal stability of chromatin and lysine copolymer–DNA complexes. They found that ethylene glycol destabilized the high-temperature melting region of the histone–DNA complexes within chromatin. Since glycol exerted only minor changes in free DNA but produced marked alteration of chromatin structure and synthetic polypeptide–DNA interactions, they suggested that glycol caused the destabilization primarily of histone–histone and histone–DNA interactions, resulting in turn in chromatin decondensation and parallel loss of the ψ-DNA contribution to the CD. The data presented herein show that the conformations of the core histone particle as well as H3–H4 and H2A–H2B are very sensitive to the addition of organic solvents.

The same CD spectra and, consequently, the same amount of secondary structure, obtained in 2.0 M NaCl at neutral pH, can be achieved in all of the complexes examined, under low-salt conditions (5.0 mM sodium phosphate and 0.05 mM DTT), with the appropriate addition of organic solvents. Upon being heated, H3–H4 and core histone complex undergo an $\alpha \rightarrow \beta$ transition in 50% ethylene glycol under low-salt conditions, while H2A–H2B undergoes an $\alpha \rightarrow$ random transition under the same conditions.

The α-helix-inducing ability of weakly protic solvents such as ethylene glycol and glycerol has been reviewed by Singer (1962). Increased helical content of proteins dissolved in aqueous mixtures containing weakly protic solvents is attrib-

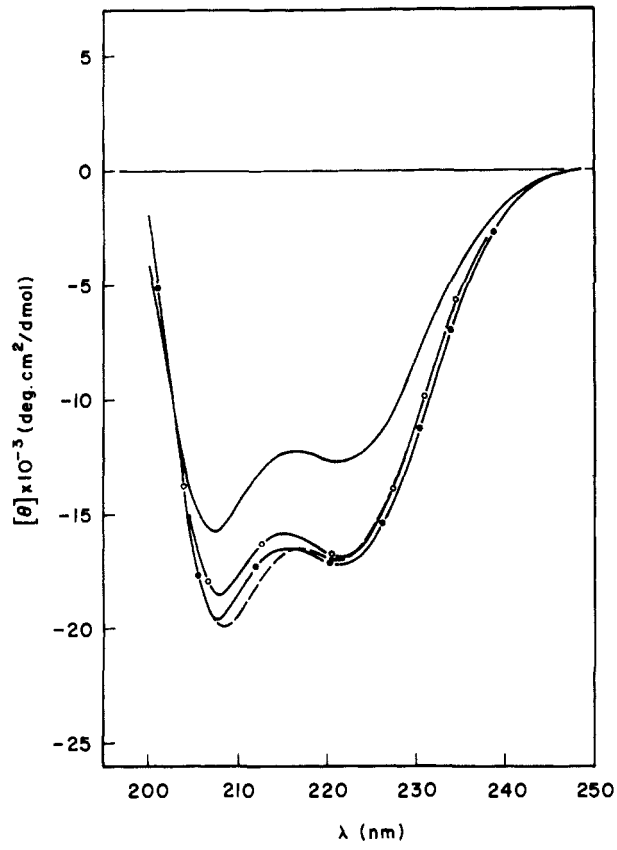

FIGURE 8: Peptide CD spectra of core histone complex under various solvent conditions. Spectra were recorded as in the legend to Figure 6, with histone concentrations from 0.35 to 0.37 mg/mL. Solvent conditions: (a) 5.0 mM sodium phosphate and 0.05 mM DTT, pH 7.0 (—); (b) as in (a) with 50% glycerol added (●); (c) as in (a) with 50% ethylene glycol added (---); (d) 2.0 M NaCl, 10.0 mM sodium phosphate, 0.10 mM DTT, pH 7.0, and 0.61 mg/mL histone (○).

uted to a decrease in the hydrogen-bonding capacity of the organic solvent compared to that of water, thereby favoring intrapeptide bond formation (Tanford, 1962), as well as decreased intramolecular electrostatic repulsion in the organic solvent due to increased counterion binding. Formation of multisubunit complexes might also allow formation of stable secondary structure, due in part to reduced hydration along the contact surface.

It might be expected, at first, that electrostatic destabilization of histone structure caused by the high charge density of the N-terminal regions would be exaggerated at increased organic concentrations, due primarily to the expected decrease in the dielectric of the environment. However, ion pairing between solute (protein) and solvent counterions can contribute to structure stabilization (Singer, 1962) in a low dielectric environment. In the absence of such shielding, e.g., at low salt, with no added organics, histone secondary structure becomes unstable.

Tanford (1968) pointed out that for most proteins, conformational changes occur only at ethylene glycol concentrations in excess of 50% by volume. Lysozyme undergoes the first distinguishable changes in its secondary structure only at an ethylene glycol concentration in excess of 80% (Ikeda & Hamaguchi, 1970). β-Lactoglobulin begins to change conformation only at 70% glycol (Tanford et al., 1962). In this respect, it is interesting that all three histone complexes studied begin to take up additional α-helical structure with as little as 10% added glycol. This is probably a consequence of the high charge density of the histones and may be related to the fact that, at low ionic strength, all three complexes have very little secondary structure.

The effect of HFP on histone secondary structure is quite dramatic. It can be estimated from Figures 6 and 7 that in about 2% HFP (~0.2 M) the histone mixtures will regain α-helical structure comparable to that induced in 2 M NaCl. The dielectric constant of HFP was not available in the literature; however, a reasonable estimate based on 2-propanol (ϵ = 18.3 at 25 °C), 1,1-dichloro-2-propanol (ϵ = 12.0 at 20 °C), and 1,1,2,2-tetrafluoro-1,2-dichloroethane (ϵ = 2.26 at 25 °C) (Kaye & Laby, 1973) is approximately 5–10. This is considerably lower than the dielectric of water (ϵ = 80 at 20 °C) as well as that of ethylene glycol (ϵ = 37.7 at 25 °C) or glycerol (ϵ = 42.5 at 25 °C) (Singer, 1962). Hence, a reduction in the dielectric of the environment may be a factor in the α-helix-inducing ability of HFP. Preferential binding to the proteins may also play a role. Parrish & Blout (1971) examined the CD spectra of five synthetic polypeptides dissolved in HFP and found that all except one were predominantly α helical. Small differences in spectral shape, very similar to those found in the histones at different HFP levels (Figure 5), were attributed to slight variations in the ϕ,ψ conformational angles. Similarly, increasing concentrations of HFP caused increased helical contents in the histone complexes.

Organic solvents may also affect intersubunit interactions. In addition to stabilizing hydrogen bonds, ethylene glycol may also disrupt hydrophobic interactions (Nozaki & Tanford, 1965). For example, at 50% ethylene glycol, lactate dehydrogenase undergoes partial but incomplete dissociation (Millar, 1974). However, the observation that similar amounts of secondary structure are found in high salt or in organic–water mixtures suggests that similar subunit interactions may occur. The observation that a high-salt environment stabilizes the association of the histone pairs to the core octamer (Eickbush & Moudrianakis, 1978) suggests that at high salt, proper secondary structure is maintained at the intersubunit binding surfaces and electrostatic repulsions are minimized. Eickbush & Moudrianakis (1978) concluded from the temperature dependence of octamer dissociation that hydrogen bonding plays an important role in stabilizing the octamer. If the primary effect of HFP at low concentration (~2%) is to reduce unfavorable electrostatic interactions by counterion pairing, thereby allowing the formation of stable high-salt-like forms of the histone pairs, without significantly altering hydrophobic or hydrogen bonds, then reassociation to the octamer may be possible at low salt by addition of low levels of HFP. Higher levels of ethylene glycol or glycerol may produce the same effect.

Transitions of α-helical regions into β sheet have been observed in proteins and synthetic polypeptides and are often thermally induced (Fasman, 1967). The $\alpha \rightarrow \beta$ thermal transition of poly(L-lysine) (at pH 11.2) is well-known and has been used in the formulation of reference CD spectra for pure β-sheet conformation (Greenfield & Fasman, 1969). Aggregation and subsequent precipitation often accompany $\alpha \rightarrow \beta$ transitions, making them difficult to study. In 50% ethylene glycol, H3–H4 undergoes a partial $\alpha \rightarrow \beta$ transition at ~23 °C, involving about 20% of the secondary structure (Figure 2), with no noticeable change in turbidity. However, in 0.25 M NaCl, 10 mM sodium phosphate, and 0.25 mM EDTA, H3–H4 becomes visibly turbid when heated above 30 °C (unpublished data), suggesting that a similar $\alpha \rightarrow \beta$ transition occurs but leads to aggregation in the nonorganic buffer. Since it appears that the same transition occurs at low temperature in both solvents, the transition may play an important role in

nucleosome activity at physiological temperature. Fasman et al. (1977), in predicting the secondary structures of the histones, found that all four inner histones had regions with both high α-helical and high β-sheet potential. In H3 and H4, these are found predominantly in the central, hydrophobic regions. This further suggests that, if $\alpha \rightarrow \beta$ interchange is indeed a significant physiological function of H3–H4, it affects intersubunit interactions.

When the core histone particle was heated in 50% ethylene glycol, a similar $\alpha \rightarrow \beta$ transition occurs, with $T_m \simeq 40$ °C. However, the spectral changes with increasing and, especially, decreasing temperatures are not the weight average of the corresponding spectral changes observed for H3–H4 (irreversible $\alpha \rightarrow \beta$) and H2A–H2B (reversible $\alpha \rightarrow$ coil). Instead, the transition is completely irreversible. This implies that an interaction between H2A–H2B and H3–H4 has taken place during the transition, locking the entire particle into an irreversible high β-sheet form. The increased T_m ($\sim$40 °C) compared to that of H2A–H2B (37 °C) and H3–H4 (23 °C) supports this idea.

The effect of $NaClO_4$ on H2A–H2B was not as expected. Peggion et al. (1972) studied the effect of perchlorate on poly(L-lysine) and found that essentially 100% α-helical content was induced at 1.0 M. Upon dialysis of H2A–H2B from 2 M NaCl, 10 mM sodium phosphate, and 0.25 mM EDTA into a 1.0 M $NaClO_4$ solution, the high-salt CD spectrum was retained. Although additonal study is required at different perchlorate concentrations, the present data suggest that the high-salt form of H2A–H2B resists further secondary structure formation in $NaClO_4$, i.e., that this form is a particularly stable one. H3–H4 precipitated during dialysis into perchlorate and could not be studied.

During the cell cycle, DNA undergoes self-replication, transcription, compaction, extension, and other processes necessary for proper cellular function. Many of these activities must be regulated, at least in part, at the nucleosome level. As such, the nucleosome can itself be considered as having an "activity", responsive to the structural requirements of the various DNA activities. One of the ways in which specifically directed histone modification might influence nucleosome activity is by altering the histone–histone and histone–nonhistone protein interactions within the nucleosome, resulting in a change in the shape of the nucleosome and, consequently, the accessibility of nucleosomal DNA to polymerases and other DNA binding proteins. Studies of the structural differences between active and inactive genes and of the binding properties of enzymatically modified histone subunits have attempted to bring the examination of nucleosome activity to the molecular level. In this study, we have begun to examine the influence of environment on the structure of the nucleosome core proteins. The data show that the core has the potential for significant changes in both α-helical and β-sheet structure and that a small change in the polarity of the environment within and around the nucleosome may greatly affect its internal structure.

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Histone Acetylation and the Deoxyribonuclease I Sensitivity of the *Tetrahymena* Ribosomal Gene†

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ABSTRACT: Under appropriate conditions, up to 85% of the total acetate can be removed from the histones of isolated *Tetrahymena* macronuclei by an endogenous histone deacetylase activity. After in vitro deacetylation, the ribosomal genes are still preferentially digested by DNase I. These observations suggested that either the majority of histone-bound acetate is unnecessary to maintain the DNase I sensitive state or ribosomal chromatin (rChromatin) histones remain acetylated under these conditions. The characteristics of histone acetylation were studied in *Tetrahymena* rChromatin, which can be isolated in a relatively pure form. Histones associated with the presumably active, DNase I sensitive ribosomal genes have a high steady-state level of histone acetylation which, surprisingly, is maintained by very low acetate turnover rates.

Most or all eucaryotic nuclear DNA, including transcriptionally active and inactive genes, is probably packaged with the four core histones (H2A, H2B, H3, and H4) into nucleosomes [for a review, see McGhee & Felsenfeld (1980)]. While the primary sequences of these histones have been highly conserved during evolution [see Isenberg (1979)], they undergo a variety of postsynthetic modifications. Since a variable fraction of potential modification sites (many of which are also highly conserved) is modified at any one time, considerable heterogeneity of nucleosomal histone may thus be generated. It is likely that this heterogeneity in some way alters the structural states of the nucleosomes, resulting in different functional states of chromatin.

The most extensively studied secondary modification of histones, acetylation, occurs at specific lysine residues near the NH_2 terminus of the four core histones [for a review, see Allfrey (1977)]. It has been postulated that this modification might weaken both histone–DNA and higher order nucleosome–nucleosome interactions, thereby making specific DNA sequences more accessible for transcription or replication (McGhee & Felsenfeld, 1980; Shewmaker & Wagner, 1980; Bode et al., 1980). Positive correlations exist between histone acetylation and transcriptional activity in a number of biological systems [see Allfrey (1977)]. Moreover, highly acetylated histones are solubilized rapidly by DNase I under conditions in which transcriptionally active chromatin is preferentially cleaved (Nelson et al., 1978a, 1979; Vidali et al., 1978; Sealy & Chalkley, 1978). Chromatin derived from cells grown in sodium butyrate, a deacetylase inhibitor (Candido et al., 1978; Boffa et al., 1978; Cousens et al., 1979), shows both an enhanced level of histone acetylation and an

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