The Histone Octamer, a Conformationally Flexible Structure†

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ABSTRACT: The conformation of the histone octamer complex in solution has been shown, by circular dichroism studies, to be highly dependent on the nature of the salt milieu and its concentration. In 2 M NaCl, the complex has 43.5% α-helix, 16% β-sheet, and 40.5% random structure. In 2.3 M $(NH_4)_2SO_4$, the octamer has 49.0% α-helix and 51% random structure. These results partially explain the discrepant results obtained by the X-ray analysis of crystals obtained under varying conditions.

The nucleosome core particle, the elementary subunit of chromatin structure (Thomas & Kornberg, 1975), contains a central octameric "core" of histones around which the nuclear DNA is wound (Thomas & Kornberg, 1975; Kornberg & Thomas, 1974) The octamer is composed of two kinds of subunits, two H2A–H2B dimers and one H3–H4 tetramer. Extensive digestion of chromatin with micrococcal nuclease releases a nucleosome core particle composed of the histone octamer and 146 ± 2 base pairs of DNA (Noll & Kornberg, 1977).

Recently, there has been a lively discussion concerning the crystallographic structures of the octamer histone core alone and in the nucleosome (Klug et al., 1985; Moudrianakis et al., 1985a,b; Uberbacher & Bunick, 1985a). Results from X-ray diffraction studies at a resolution greater than 3.3 Å (Burlingame et al., 1984, 1985), have been presented on the isolated histone octamer. The interpretation of these results differed significantly in the shape, size, and internal arrangement from that suggested from a crystallographic analysis of nucleosome core particles (Finch et al., 1977; Richmond et al., 1984). Uberbacher and Bunick (1985a) have studied a nucleosome core particle by X-ray diffraction and have stated "we are having considerable difficulty rationalizing the interpretation of the histone octamer of Burlingame et al. (1985) with the nucleosome structure we have solved (Uberbacher & Bunick, 1985b), with those of Richmond et al. (1984) and Bentley et al. (1984), and with many other biophysical studies". In 1986, Uberbacher et al. (1986) studied the histone octamer by small-angle neutron scattering and drew conclusions that differed from those of Burlingame et al. (1984, 1985). In this paper evidence is presented that partially moderates these disagreements and concludes that each group of researchers could be correct as each is working under different distinctive conditions.

The literature on chromatin has a long and controversial history, which is filled with numerous contradictions. However, few authors would disagree with the proposition that chromatin conformation undergoes numerous changes during the various biological events that occur in the nucleus. Thus replication, transcription, cell division, etc. would probably be accompanied by significant changes in the organization of chromatin.

Biophysical studies [e.g., see reviews by Allfrey (1980) and Fasman (1978)] have shown that small changes in ionic strength can cause large changes in chromatin conformation, and different ionic environments can cause perturbation of the structure. This paper contains data obtained by measurement of the circular dichroism (CD)[1] spectrum of the histone octamer under varying conditions selected from the above listed papers. Previously, it has been shown that the histone octamer can undergo conformational changes, as measured by circular dichroism (Beaudette et al., 1981, 1982), as a function of the surrounding media. Further, it was demonstrated by high-performance liquid chromatography, sedimentation equilibrium, and circular dichroism studies that the association of the histones and their conformation in the octamer are affected by a variation of ionic strength and the concentration of the histones (Prevelige, 1985; Prevelige & Fasman, 1987).

Thus, if the octamer histone core and the nucleosome are viewed as a very pliable structure, it is not surprising to find different conformations occurring, locked temporarily by the local milieu. Viewing these contradictory papers in this light allows for more insight into the extremely perturbable chromatin conformation.

Experimental Procedures

Histone Purification. Nuclei of chicken erythrocytes were prepared from chicken blood (Pel Freeze Biochemicals) by the method of Ramsay-Shaw et al. (1976). The red blood cells were broken by freezing and thawing, and nuclei were isolated by centrifuging in saline–citrate (0.15 M NaCl and 15 mM sodium citrate at pH 7.2) solution. From the nuclei, chromatin core particles are isolated by the method of Fulmer and Fasman (1979), in which the nuclei were digested by micrococcal nuclease (from Pharmacia Biochemicals, lot NE58429). The chromatin was isolated by centrifugation and then extracted in 0.25 mM EDTA, followed by centrifuging, to yield a soluble chromatin supernatant. Histones H1 and H5 were stripped from the core particles by treatment with Dowex AG-50 W-X2 resin (Bio-Rad), according to the methods of Bolund et al. (1973) and Thoma and Koller (1977). To isolate the histone octamer, DNA was removed by eluting through a hydroxyapatite (Bio-Gel HTP, DNA grade, from Bio-Rad, lot 23314) column in 2.0 M KCl buffer (Rhodes, 1979). After purification, the histone octamer was dialyzed into 2.0 M NaCl buffer, which contains 10 mM sodium phosphate, 25 mM Na_2EDTA, 0.1 mM DTT, and 0.1 mM PMSF at pH 7.2, and

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[1] Abbreviations: CD, circular dichroism; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate.

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stored frozen at −70 °C. The purity of the octamer was confirmed by 15% polyacrylamide–SDS gel electrophoresis in the Laemmli (1970) system. Up to the limit of the sensitivity of the silver-staining method, which has been known to detect proteins in the nanogram level (Wray et al., 1981), there were only the four histone bands found (data not shown).

Preparation of Histone Solutions in Various Ammonium Sulfate Concentrations. Histone solutions in specific concentrations of ammonium sulfate were made by dialysis using previously washed dialysis tubing (Spectrapor 3, Spectrum Medicals, Inc.). The dialysis was performed with histone solutions of less than 1 mL in 2.0 M NaCl buffer, against 1 L of dialysis buffer, which contained 10 mM sodium pyrophosphate, 5 mM Na_2EDTA, and 0.1% 2-mercaptoethanol in addition to ammonium sulfate at the required concentration, for 48 h with two changes of the buffer–ammonium sulfate mixture.

Burlingame et al. (1984) used 1% 2-mercaptoethanol to crystallize the protein rather than the 0.1% used herein. However, for the optical measurements, such as circular dichroism and especially UV absorption spectra, the higher content of the 2-mercaptoethanol had a very high absorbance at 260 nm, so that decreasing its concentration to 0.1% allowed more accurate measurements to be made.

Determination of Histone Concentration. The concentration of the histone octamers in the individual solutions was determined, after dialysis, by UV absorption spectra. Any precipitation of the protein could easily be detected by its scattering of light. $E_{277}^{1\%,1cm} = 4.64$ (McCarthy et al., 1984) was used for calculation of the concentration by UV absorbance measurements.

Circular Dichroism Measurement. CD spectra of histone solutions in various concentrations of ammonium sulfate were obtained from 0.5 to 3.0 M in increments of 0.5 M. Higher than 3.0 M concentratons of ammonium sulfate caused precipitation of the majority of the protein. Therefore, a lower range of the ammonium sulfate concentrations, i.e., from 0.5 to 2.0 M, was used. CD spectra were taken in the wavelength range of 200–300 nm. It was thus possible to detect any scattering at wavelengths greater than 250 nm and to discard such measurements.

Since the crystals were obtained at 2.3 M ammonium sulfate (personal communication from Uberbacher and Bunick), our measurements were made in the concentration range of 2.0–2.5 M in increments of 0.1 M. To prevent crystallization, a lower concentration of histones (0.1–0.3 mg/mL) was used. Each spectrum reported was obtained by averaging at least four spectra for each concentration of ammonium sulfate (for the experimental conditions for the spectra, see the legend in Figure 1). The error bars in Figure 2 represent the standard deviatons of individual values from the average.

After the CD measurements, the solutions were electrophoresed (Laemmli, 1970) to see if any degradation had occurred during the measurement. No detectable degradation was observed by the silver-staining method (Wray et al., 1981).

Results

The histone octamer obtained from chicken erythrocytes was prepared as previously described (Beaudette et al., 1981). The circular dichroism spectra were obtained with a Jobin-Yvon Mark V autodichrograph (Prevelige & Fasman, 1987). Two different ionic conditions were investigated. The first was similar to that used by Burlingame et al. (1984, 1985) [i.e., 2.0–2.5 M $(NH_4)_2SO_4$] and contained the octamer but at a lower protein concentration than used for crystal formation (20 mg/mL). A stock solution of histone octamer, in 2.0 M NaCl, 10 mM sodium phosphate, pH 7.2, 25 mM EDTA, 0.1 mM DTT, and 0.1 mM PMSF, was dialyzed (with washed dialysis tubing Spectrapor 3, Spectrum Medicals, Inc.) vs various concentrations of ammonium sulfate, 10 mM sodium pyrophosphate, pH 7.2, 5 mM EDTA, and 0.1% 2-mercaptoethanol. The second condition investigated was that of Uberbacher et al. (1986), namely, 2 M NaCl. Unfortunately, it was impossible to study the histone octamer under the conditions of Finch et al. (1977) or Richmond et al. (1984), as the exact conditions used for crystallization were not published.

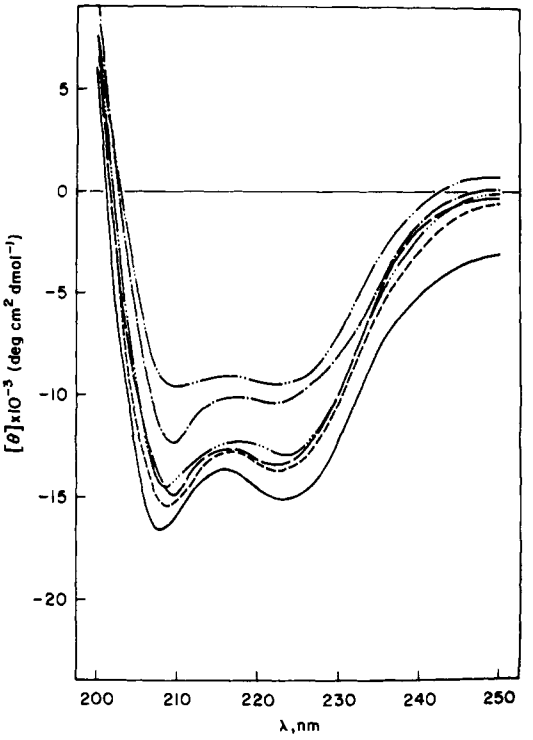

FIGURE 1: Circular dichroism spectra of the histone octamer core in various concentrations of $(NH_4)_2SO_4$: 2.0 M (—); 2.1 M (---); 2.2 M (-·-); 2.3 M (— —); 2.4 M (-···-); 2.5 M (-··-). Concentration of protein 0.1–0.3 mg/mL; path length 0.02 cm; temperature 22–24 °C; number of scans 20.

Circular Dichroism Studies. The CD spectra of the histone octamer as a function of $(NH_4)_2SO_4$ concentration are seen in Figure 1. A summary of these data, $[\theta]_{222}$ and $[\theta]_{208}$ vs $[(NH_4)_2SO_4]$, is found in Figure 2. A very interesting observation was recorded. The negative ellipticity decreases in magnitude, then increases, and again decreases with increasing concentration of $(NH_4)_2SO_4$. The behavior of the octamer as a function of [NaCl] is seen in Figure 3. [This is reproduced from Prevelige and Fasman (1987).] The negative ellipticity at $[\theta]_{208}$ and $[\theta]_{222}$ increase in a linear manner with increasing NaCl concentration, which is quite distinctively different from the behavior observed in $(NH_4)_2SO_4$. At lower concentrations of $(NH_4)_2SO_4$ (namely 0.5–2.0 M), the ellipticity values at 208 and 222 nm and the shape of the spectra did not change significantly from that at 2.0 M.

The secondary conformation of the histone octamer complex in the various salt concentrations was calculated from the circular dichroism curves, according to the Yang et al. (1986) procedure, and is found in Table I.

Although the exact interpretation of the secondary structure derived from circular dichroism curves is open for discussion (Yang et al., 1987; Woody, 1985), nevertheless, within one method *relative differences are significant.* This is the important factor in the present analysis, where *significant* dif-

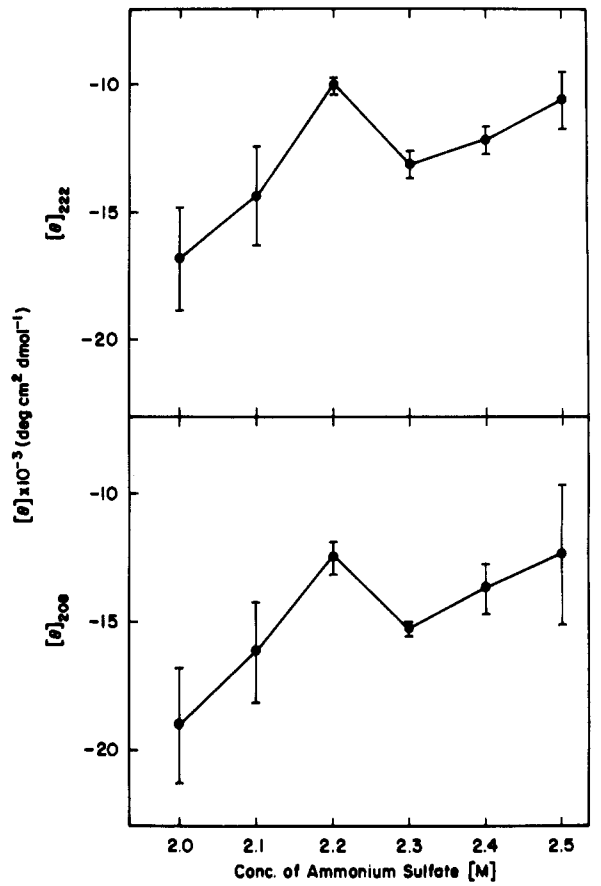

FIGURE 2: Circular dichroism ellipticity values $[\theta]_{208}$ and $[\theta]_{222}$ of the histone octamer core vs $(NH_4)_2SO_4$ concentrations. Conditions as described in Figure 1.

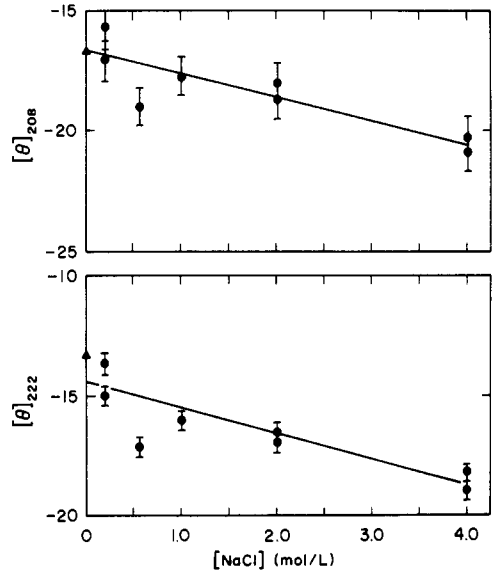

FIGURE 3: Circular dichroism ellipticity values $[\theta]_{208}$ and $[\theta]_{222}$ of the histone octamer core vs NaCl concentration. Protein concentration range 0.18–0.25 mg/mL; path length of cell 0.05 cm (Prevelige & Fasman, 1987).

ferences were found. Thus as the NaCl concentration increases, the α-helical content of the octamer increases, while the β-structure decreases and the random regions remain similar. However, a quite strikingly different behavior is observed with $(NH_4)_2SO_4$. The α-helical content of the octamer decreases on going from 2.0 to 2.2 M $(NH_4)_2SO_4$, then increases slightly at 2.3 M, and again decreases up to 2.5 M $(NH_4)_2SO_4$. No explanation for this behavior is evident. Both

Table I: Estimates of the Secondary Conformation of the Histone Octamer Complex in Various Concentrations of $(NH_4)_2SO_4$ and NaCl Using the Yang et al. (1986) Method[a]

buffers	μ[d]	α-helix (%)	β-sheet (%)	random (%)
0.2 M NaCl[b]	0.2	38.5 ± 3.4	19.0	42.5
2.0 M NaCl	2.0	43.5 ± 2.7	16.0	40.5
4.0 M NaCl	4.0	54.0 ± 2.9	5.0	39.5
2.0 M $(NH_4)_2SO_4$[c]	6.0	50.5 ± 5.9	0	49.5
2.2 M $(NH_4)_2SO_4$	6.6	45.0 ± 2.2	0	55.0
2.3[e] M $(NH_4)_2SO_4$	6.9	49.0 ± 1.0	0	51.0
2.5 M $(NH_4)_2SO_4$	7.5	41.0 ± 8.9	0	59.0

[a] The β-bend values determined by this method have been incorporated into the percent random coil. [b] The buffers also include 10 mM sodium phosphate, 25 mM EDTA, 0.1 mM DTT, and 0.1 mM PMSF at pH 7.2. [c] The buffers also include 10 mM sodium pyrophosphate (pH 7.2), 5 mM EDTA, and 0.1% 2-mercaptoethanol. [d] $\mu = {}^1/_2\sum c_i z^2_i$. [e] The concentration stated by Uberbacher et al. (1986) as 3.5 M $(NH_4)_2SO_4$ was 2.3 M $(NH_4)_2SO_4$ (communication from the authors).

$[\theta]_{208}$ and $[\theta]_{222}$ are indicators of the presence of the α-helical structure (Greenfield & Fasman, 1969).

Therefore, the crystals obtained from the solution in 2.3 M $(NH_4)_2SO_4$ (Burlingame et al., 1985) were derived from an octamer solution conformation of 49.0% α-helix and 51.0% random coil, while the study performed on the octamer solution in 2.0 M NaCl (Uberbacher & Bunick, 1985b) was derived from a solution conformation of 43.5% α-helix, 16% β-sheet, and 40.5% random coil. The analysis of Yang et al. (1986) also yields estimates of β-bends. This value has been included herein in the random regions, as recent studies have shown (M. Hollosi et al., 1985, 1987a,b) that there are several β-bend circular dichroism spectra that have not been utilized in the Yang procedure. The accuracy of the α-helical content is the highest and is independent of the values obtained for the other secondary structures (Hennessey et al., 1981).

The nucleosome core particle studied by Uberbacher and Bunick (1985b) was crystallized with various amounts of spermine, NaCl, and buffer. But the precise conditions were not stated. The crystals of the nucleosome core particle studied by Finch et al. (1977) were obtained from solutions containing "spermine tetrahydrochloride, or 3–4 mM $MgCl_2$ plus 0.2 mM spermine, or else at 10 mM $MgCl_2$, 1.5 mM spermine, and NaCl up to concentrations of 100 mM". Thus the conditions of crystallization of the nucleosome core particles used by various authors are incompletely described. If the solution structure of the octamer varied, it is likely that the crystals so obtained are likewise different.

Discussion

This paper adds further evidence to the small-angle neutron scattering study of Uberbacher et al. (1986), which demonstrated that the histone octamer complex in solution exists in different conformations, depending on the specific salt environment. Herein, this is again demonstrated as well as the fact that the conformation is also dependent on the concentration of the particular salt used. The study by McCarthy et al. (1984) had previously demonstrated the effects of ionic strength and state of assembly on the kinetics of hydrogen exchange of calf thymus histones.

Two studies (Eickbush & Moudrianakis, 1978; Benedict et al., 1984) have attempted to discern the factors responsible for the histone core complex assembly. Two sets of protein–protein interactions were held responsible for assembly. These were (1) mostly hydrophobic interactions that yield the H2A–H2B dimer and the H3–H4 tetramer and (2) interactions involving the weak association of one H3-H4 tetramer with two H2A–H2B dimers to form an octamer, which were suggested to be predominantly from histidine–lysine or his-

tidine–tyrosine hydrogen bonds between the dimer and tetramer units. In a recent paper, Benedict et al. (1984) performed a calorimetric study of the octamer assembly and showed that the two-step assembly of octamer in high salt (2 M NaCl) was accompanied by large unequal enthalpies and concomitant proton release. The equilibrium constants derived agree well with a previous study (Godfrey et al., 1980). The large exothermic heats (Benedict et al., 1984) associated with the histone assembly reactions are consistent with a dominance of H-bonding (Ackers, 1980; Ross & Subramanian, 1981), as suggested previously by Eickbush and Moudrianakis (1978).

The salt effects noted herein would be expected to weaken attractive or repulsive inter- and intrachain charge–charge interactions and to have specific effects on conformational stability and on association–dissociation equilibria in a manner that has been thoroughly investigated but only partially understood (von Hippel & Schleich, 1969). Two fundamentally different classes of mechanisms can be formulated to account for ion-induced effects on macromolecular stability: direct ionic interactions with macromolecules and indirect interaction transmitted by solvent structure changes induced by the added ions (von Hippel & Schleich, 1969). This intricate subject cannot be discussed herein; however, the ionic effects observed for the histone core complex can be attributed to such considerations. If the transconformational transitions are delicately balanced, then it is not surprising to find significant differences in structure upon altering the ionic media of the histone core complex. To quote Benedict et al. (1984), "The involvement of a limited number of H-bonds in the dimer–tetramer assembly offers a high degree of specificity in the contact of these interfaces, while also keeping the energetic requirements of the reversibility to a minimum."

Thus it is not surprising that the conformation in 2 M NaCl (μ = 2.0) and that in 2.3 M $(NH_4)_2SO_4$ (μ = 6.9) are different.

It has been demonstrated in this paper that the conformational changes in chromatin required to enable the various biological functions to be performed can be brought about by minor changes in the environment of the nucleus.

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