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ON THE NATIVE STRUCTURE OF THE HISTONE H3-H4 COMPLEX

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SUMMARY

Electrophoretic and sedimentation velocity studies on the histone H3-H4 complex show that provided the H3 cysteine residues remain reduced the complex reforms quantitatively when removed from a variety of denaturing conditions. If histone H3 is allowed to become intramolecularly oxidized while denatured only monomer and large aggregates are formed on return to native conditions. At pH 7 ionic strength 0.1 the complex remains with reduced sulfhydryl groups indefinitely suggesting a vital role for the sequence 96-110 in histone H3 in the tertiary structure of the complex.

Histones H3 and H4 have been shown (1-3) to form a tetrameric complex in solution and indirect evidence (4-6) has been presented which indicates that these two histones are associated in chromatin. Although structural details of this complex have yet to be elucidated there appears to be a controversy in the literature (1, 2, 6-8) as to whether or not the complex as extracted from chromatin has a tertiary or quanternary structure that can be irreversibly altered by extremes of pH, temperature or the presence of denaturants.

I wish to report the results of some experiments carried out with chromatin extracted complexes which suggest that the "nativity" of the complex in solution depends exclusively on the cysteine residues being reduced. No evidence for an irreversibly altered structure has been found when only noncovalent structural changes occur in these two histones.

MATERIALS AND METHODS

Whole histone was extracted from calf thymus chromatin (9) by protamine extraction (10) and was further fractionated (10) to yield a pure histone H3-H4 complex. The urea-acetic acid gels of Panyim and Chalkley (11) were used for identifying the respective histones and for determining (12) the extent of oxidation of the histone H3 cysteine residues. The double gel was run according to Lewis et al. (13).

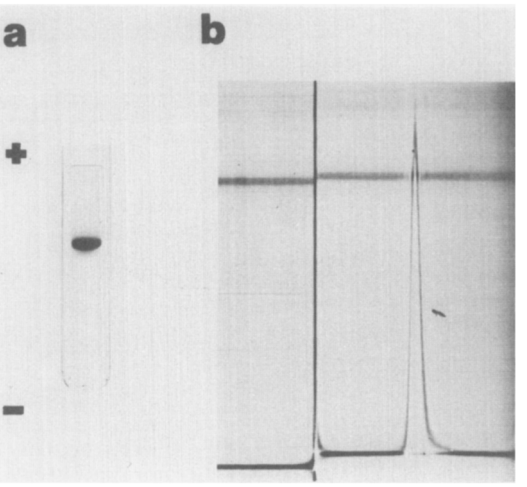

Figure 1. (a) Electrophoresis and (b) sedimentation of the histone H3-H4 complex extracted from calf thymus chromatin. Electrophoresis was carried out on 0.05M phosphate pH 7 polyacrylamide gels for 6 hrs. at 100 volts, 30 μg of protein were loaded. The gels were stained with amido black. The same protein sample was sedimented in a synthetic boundary cell at 20,000 rpm. The schlieren pattern shown was recorded as soon as the rotor reached speed.

The existence of a complex was monitored where indicated by sedimentation velocity measurements run at 20°C (5mgs/ml protein) in a Beckman Model E analytical ultracentrifuge equipped with schlieren optics and by electrophoresis on nondenaturing 10%, 0.05 M phosphate pH7 polyacrylamide gels (14) with cooling and buffer recirculation facilities. The various conditions to which the complex has been exposed are indicated in the next section.

RESULTS AND DISCUSSION

The histone H3-H4 complex as isolated from calf thymus chromatin migrates as a single band on nondenaturing 0.05M phosphate pH7 polyacrylamide gels and also when sedimented at 20°C as shown in Figure 1 (a) and (b) respectively. The chromatin extracted complex was found to have an S value of 2.6 in reasonable agreement with the data of other investigators (1,8). There was virtually no (<5%) high molecular weight material in our sample in direct contrast to work of Sperling and Bustin (8) who report that more than 50% of the protein

Table I - Effect of Various Solvents on the Reformation of the Histone H3-H4 Complex as Judged by Gel Electrophoresis.

	Treatment[a]	Gel Pattern[b]	Histone H3 Sulfhydryl Condition[c]
1	8M Urea, pH 7, ionic strength 0.1, 2% ME, 20 hrs.	H3-H4 complex only	reduced
2	8M urea, pH 7, ionic strength 0.1 20 hrs.	aggregated and monomeric H3 and H4, no complex	intramolecularly oxidized
3	Treatment 2 for 20 hrs followed by addition of 2% ME for 2 hours	H3-H4 complex only	reduced
4	0.05M phosphate pH 7 aereated >100 hours	H3-H4 complex only	reduced
5	0.0001M phosphate pH 7 aereated 20 hours	aggregated and monomeric H3 and H4, no complex	intramolecularly oxidized
6	various pH values between 1 and 5 and ionic strengths between 10^{-1} and 10^{-4}, aereated for 20 hrs.	a mixture of complex, aggregate and monomeric H3 and H4	reduced and intramolecularly oxidized

(a) Treatments 1,2 and 3 were made by adding solid urea and dilute base to a 5 mg/ml solution of the H3-H4 complex in 0.05M Na acetate and 0.05M Na bisulfite pH 5.5. ME is 2-mercaptoethanol. Treatments 4,5 and 6 were made by dialyzing the above protein solution against several changes of the appropriate buffer at room temperature.

(b) 30 µl of the treated histone solution was electrophoresed onto a nondenaturing 0.05M phosphate pH 7 polyacrylamide gel for 6 hours at 100 volts. The very same results were obtained when the treated solutions were first dialyzed against the original acetate-bisulfite pH 5.5 buffer before electrophoresis.

(c) As judged by the mobility of histone H3 on urea-acetic acid gels (12).

is aggregated (S>20) under the same conditions when the complex is formed from purified histones. We have found that when either pure histone H3 or H4 is separately electrophoresed on the nondenaturing phosphate gels most but not all (>80%) of the protein remains at the origin which is consistent with the known (8) aggregating properties of these two histones at pH7 ionic strength 0.1.

The principal difference between the synthetic and chromatin extracted complex then appears to be in the extent of aggregation of a portion of the

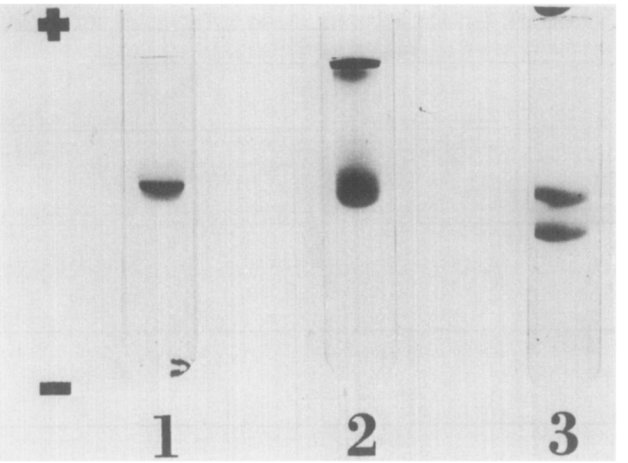

Figure 2. The effect of 8M urea on the histone H3-H4 complex. Gels 1 and 2 are of the 0.05M phosphate pH 7 type to which untreated complex and 20 hour 8M urea pH 7 treated complex were applied respectively. Electrophoresis was for 6 hrs. at 100 volts. Gel 3 is a double gel of the Panyim and Chalkley acetic acid-urea type (11), the untreated complex is on the left side and the 20 hour 8M urea pH 7 treated complex is on the right side. Electrophoresis was for 3 hours at 120 volts.

protein. To establish the basis for this difference we have subjected samples of the chromatin extracted H3-H4 complex separately to a variety of denaturing conditions and then examined by electrophoresis the molecular species formed when the native conditions were reestablished. The various treatments applied as well as a description of the resulting phosphate gel pattern and sulfhydryl oxidation state are given in Table I. The phosphate gel patterns obtained were of two basic types as shown by patterns 1 and 2 in Figure 2. Pattern 1 was obtained from the untreated chromatin extracted H3-H4 complex and pattern 2 from the same complex treated for 20 hrs with 8M urea, pH7 ionic strength 0.1. Pattern 3 in Figure 2 is a urea-acetic acid (11) double gel of the untreated complex on the left and the urea treated complex on the right. By virtue of its increased mobility the H3 histone, the slower of the two bands on the right,

is competely in the intramolecularly oxidized form (12). No H3 dimer or higher order polymers were detected when the complex was treated with 8M urea at pH7 (15). A sedimentation velocity run on the oxidized H3-H4 mixture with the urea removed indicated that more than 80% of the protein had S values greater than 25. The fast moving band in gel pattern 2 is due to uncomplexed monomeric H3 and H4 histones. This was established by electrophoresing the pure histone fractions on the same gels and gels containing 8M urea. As well as aggregates both histone H3 and H4 contribute a small amount (20%) of fast moving protein (monomeric). It can be concluded therefore that histone H3 and H4 do not interact when H3 is intramolecularly oxidized.

The effect of sulfhydryl oxidation in H3 on complexation is completely reversible. When the oxidized H3-H4 mixture was reduced in 8M urea it gave a gel pattern identical to the untreated complex as indicated by treatment 3 in Table I. Aereation of the complex at pH7 ionic strength 0.1 room temperature gave no apparent sulfhydryl oxidation after several days indicating the strength of the H3-H4 association (7). At low ionic strength at pH7 the complex is apparently unstable as eviced by treatment 5. On the basis of the treatment 6 it can be concluded that the complex becomes progressively less stable as the pH and ionic strength are reduced. Böhm et al. (16) have shown that at pH2.3 ionic strength 0.4, H3 and H4 can be completely separated by gel filtration and are therefore non-interacting. All of the treatments in Table I were allowed to remain at room temperature 20 hrs or longer before electrophoretic analysis. When the complex was treated as in Table I and electrophoresed immediately no change in the phosphate gel pattern or oxidation of H3 occurred. Similar results were found for other treatments not given in Table I such as acetone precipitation, heating and 7M guanidine HCl.

Put simply the results of this study demonstrate that provided the H3 cysteine residues are reduced, the denatured histone H3 and H4 constituents of the complex will recombine spontaneously with no aggregate formation when returned to a nondenaturing aqueous medium with pH values greater than 5 and

ionic strength 0.1 or greater. I therefore propose that the observed differences between the chromatin extracted and synthetic H3-H4 complexes are due to incomplete reduction of the H3 histone and perhaps a nonstoichiometric mixture.

The sensitivity of the H3-H4 complex formation to the existence of a disulfide bridge between histone H3 residues 96 and 110 suggests that this region might be one of the primary contact sites between the H3 and H4 histones. If the H3-H4 complex is vital to the structural integrity of chromatin then the important question arises as to whether or not it is possible to faithfully reconstitute calf thymus chromatin when H3 is intramolecularly oxidized. It is interesting to note that the histone H3 in rat and mouse liver (favourite tissues for chromatin reconstitution) has only one cysteine residue (12) and so an intramolecular disulfide bridge cannot occur and thereby prevent the H3-H4 complex from renaturing.

ACKNOWLEDGMENTS

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