A TWO-SUBUNIT HISTONE COMPLEX FROM CALF THYMUS

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Received April 8,1974

SUMMARY

Studies by equilibrium ultracentrifugation demonstrate that a complex can form between histones f2a1 and f3. This two-subunit complex undergoes a concentration-dependent reversible association to a four-subunit complex. Less than 3% separate f2a1 or f3 is present under the conditions of this investigation. Non-denaturing methods have been used to isolate the f2a1-f3 complex.

The mechanisms involved in possible structural and regulatory functions of histones may lie in the precise histone-DNA and histone-histone interactions. In elucidating these interactions, care must be taken to avoid irreversible conformational denaturation during histone isolation; and procedures commonly known to denature proteins probably should be avoided. The present study describes the existence of a two-subunit histone complex isolated from calf thymus chromatin under presumably non-denaturing conditions.

MATERIALS AND METHODS

Histone Isolation. Nuclei were isolated from calf thymus (1); and chromatin was obtained from osmotically ruptured nuclei and washed at least 6 times with a 0.05M histidine, 0.05M $NaHSO_3$, 0.015M NaCl, 0.005M EDTA, pH 6 buffer. Histones were extracted with 0.5M $MgCl_2$ (plus 0.05M histidine, 0.05M $NaHSO_3$, pH 6). The procedures of van der Westhuyzen and von Holt (2) were used to separate histones from the DNA and to fractionate the histones by chromatography in a 2.5 x 100 cm column of Sephadex G-100 superfine in 0.05M NaOAc, 0.05M $NaHSO_3$, pH 5. The leading peak was fractionated by $(NH_4)_2SO_4$ precipitation at 70% saturation to yield f1 in the supernatant and f2a1-f3 in the pellet. The f2a1-f3 fraction was essentially pure and showed no

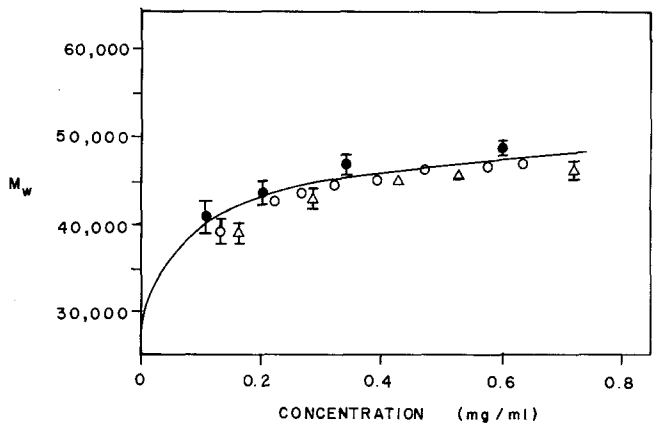

Figure 1. Association behavior for f2a1-f3. Sedimentation-equilibrium experiments under conditions: 0.05M NaOAc, 0.05M $NaHSO_3$, 0.005M EDTA, 0.02% NaN_3, pH 5; 20°C; 34,000 rpm. Weight-average molecular weight is presented as a function of local equilibrium concentration for three loading concentrations: ● 0.2 mg per ml; ○ 0.6 mg per ml; △ 1.8 mg per ml. Solid line is predicted behavior for a monomer-dimer equilibrium (M_1=27,300, K_2=1.98·10^5 liters per mole).

evidence of degradation by criteria of gel electrophoresis (2,3).

Equilibrium Ultracentrifugation. High-speed sedimentation-equilibrium experiments were performed according to the methods of Yphantis (4), Roark and Yphantis (5), and Ansevin *et al.* (6). The histone solution was dialyzed exhaustively against 0.05M NaOAc; 0.05M $NaHSO_3$, 0.005M EDTA, 0.02% NaN_3, pH 5. All experiments were performed at 20°C in a Spinco Model E Analytical Ultracentrifuge, equipped with interference optics, at rotational speeds of 34,000 rpm. Interference fringe patterns were read by an automated micro-comparator (7); and local weight-average (M_w) and Z-average (M_z) molecular weights were estimated as a function of radial position (5). Partial specific volume of 0.733 ml/gm for f2a1-f3 was estimated from the amino acid composition (8) and corrected for Donnan effect non-ideality (9).

RESULTS

Figure 1 presents results of sedimentation-equilibrium experiments on the f2a1-f3 fraction. Weight-average molecular weight is an increasing function of concentration. Experiments performed at a series of initial loading concentrations can distinguish between this increase being due to

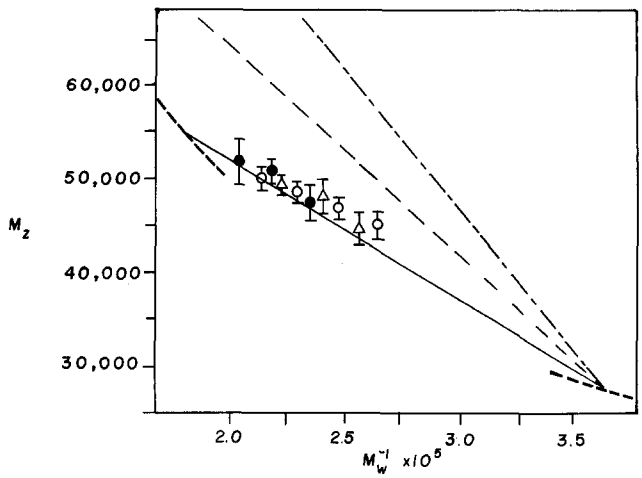

Figure 2. "Two-species plot" for f2a1-f3. The data of Figure 1 is presented with $M_z(r)$, a function of $M_w^{-1}(r)$ for three loading concentrations: ● 0.2 mg per ml; ○ 0.6 mg per ml; △ 1.8 mg per ml. Solid line represents a monomer-dimer equilibrium ($2\alpha_1\beta_1 \rightleftharpoons \alpha_2\beta_2$), M_1=27,300. Dashed and broken lines represent monomer-trimer and monomer-tetramer equilibria, respectively. Portions of the hyperbola, $M_z \cdot (1/M_w) = 1$, are shown as the heavy dashed line.

sample heterogeneity or the reversible concentration-dependent association of a single thermodynamic component (4,5). Overlap of the molecular weight averages in Figure 1, for experiments at loading concentrations of 0.2, 0.6, and 1.8 mg per ml, demonstrates that the system is essentially concentration-dependent and homogeneous. To determine what molecular-weight species are present, the sedimentation-equilibrium data of Figure 1 are presented in the form of a "two-species plot" in Figure 2 (5). If only two species, M_1 and M_2, are present, values of M_z *vs* $1/M_w$ yield a straight line which intercepts the hyperbola, $M_z \cdot (1/M_w) = 1$, at $M_z = M_1$ and M_2. Figure 2 indicates that one of the species has a molecular weight of 54,000 ± 2000. The sample contains f2a1 and f3 which have molecular weights of 11,240 and 15,320 respectively (10,11). The homogeneity demonstrated by Figure 1 indicates that the 54,000 ± 2000 molecular weight species is the oligomer of a single self-associating species. The condition of homogeneity plus the presence of the 54,000 m.w. oligomer require--and the data of Figure 2 demonstrate--that the self-associating species is the 27,000 m.w. dimer composed of one

f2a1 subunit and one f3 subunit ($\alpha_1\beta_1$). The data of Figure 2 best obey a monomer-dimer equilibrium ($2\alpha_1\beta_1 \rightleftharpoons \alpha_2\beta_2$), presented as the solid line, with a monomer molecular weight of 27,300 $\pm$ 1000. The slight deviation from this two-species line may indicate the presence of small amounts of lower molecular weight material, perhaps separate histone. If values of the reduced molecular weights or "sigmas" are assumed, a linear least-squares analysis of the concentration distribution yields the amplitudes of the corresponding exponentials. One such fit assumed the presence of three sedimenting species: separate histone of average m.w. 13,500, the 27,000 m.w. f2a1-f3 dimer, and the 54,000 m.w. tetramer. This fit demonstrated that, at most, only 3% of the f2a1-f3 is present as separate histone. Least-squares analysis, assuming only the dimer and tetramer to be present, was also used to estimate the standard free energy of association. These fits resulted in variances of only 2 microns of fringe displacement (0.002 mg per ml) indicating the data could be well represented by an equilibrium between these two species. The standard free energy of association for the $2\alpha_1\beta_1 \rightleftharpoons \alpha_2\beta_2$ equilibrium was -3.6 $\pm$ 0.2 kcal/(mole $\alpha_1\beta_1$). The solid line of Figure 1 is the predicted value of M_w using this ΔF°.

DISCUSSION

Histone purification procedures may lead to permanent disruption of the native conformation and, hence, of the histone interactions. Treatment with high concentrations of GuHCl or urea should result in some unfolding of the native structure. Treatment by non-polar solvents should cause some inversion of the protein conformation with previously buried hydrophobic residues now exposed to the solvent. Removal of the organic solvent may fail to result in a reversion to the original conformation, with the proteins continuing to have an increased number of exposed hydrophobic groups causing increased aggregation and reduced solubility at high ionic strengths. We have conducted experiments designed to detect irreversible conformational changes. Salt-extracted histone samples were initially examined by ORD and

by sedimentation-equilibrium to determine the percent α-helix and aggregate interactions, respectively, under the reference conditions: 0.05M NaOAc, 0.05M $NaHSO_3$, pH 5. The sample was then subjected to a condition under question for several hours and then redialyzed into the reference solvent. ORD and sedimentation-equilibrium measurements were repeated. Any change in these measurements indicated an irreversible alteration of the conformation. Conditions examined included 80% acetone; 80% ethanol plus 1.25N HCl; 0.01N HCl; 40% GuHCl; 10M urea; or extraction of chromatin with H_2SO_4 followed by precipitation with ethanol. Not surprisingly, these conditions led to irreversible changes in helix content of at least 10% and to increased tendencies to aggregate. A future publication will detail these and additional conformational studies. The salt-extracted histones, on the other hand, showed no decrease in solubility at high ionic strengths nor any tendency to form gross aggregates (as shown by sedimentation-velocity experiments). This behavior is contrary to that found using histones prepared by other means (12,13,14). Any irreversible conformational changes occuring during histone isolation may reduce the biological significance of previously reported histone complexes (15,16,17,18).

The work presented here suggests 1) that f2a1 and f3 can form a two-subunit homogeneous species of molecular weight 27,000; 2) that under conditions reported here, less than 3% of the subunits exist separately; and 3) that this 27,000 m.w. species undergoes a concentration-dependent reversible association to a four-subunit species containing two f2a1 and two f3 subunits. We propose that the two-subunit complex reported here be termed h3. This complex has at least two polar regions: the N-terminal segments of both subunits (10,11). If the complex exists as such *in vivo*, these polar regions may interact with DNA to form a histone bridge which may be responsible for some structural aspects of the nucleohistone complex.

ACKNOWLEDGEMENTS

This work supported in part by USPHS, NIH grants GM18456 and RR-576.

REFERENCES

1. Wang, T. Y. in *Methods in Enzymology*, vol. 12A, ed. Grossman, L. and Moldave, K. (Academic Press, New York) pp. 417-421 (1967).
2. van der Westhuyzen, D. R. and von Holt, C., *FEBS Letters* 14, 333 (1971).
3. Panym, S. and Chalkley, R., *Arch. Biochem. Biophys.* 130, 337 (1969).
4. Yphantis, D. A., *Biochemistry* 3, 297 (1964).
5. Roark, D. E. and Yphantis, D. A., *Ann. N.Y. Acad. Sci.* 164 (Art 1), 245 (1969).
6. Ansevin, A. T., Roark, D. E. and Yphantis, D. A., *Analyt. Biochem.* 34, 237 (1970).
7. Carlisle, R. M., Patterson, J. I. H. and Roark, D. E., *Analyt. Biochem.*, in press.
8. Haschemeyer, R. H. and Haschemeyer, A. E. V., *Proteins* (John Wiley, New York), p. 162 (1973).
9. Roark, D. E. and Yphantis, D. A., *Biochemistry* 10, 3241 (1971).
10. Delange, R. J., Fambrough, D. M., Smith, E. L. and Bonner, J., *J. Biol. Chem.* 244, 319 & 5669 (1969).
11. Delange, R. J., Hooper, J. A. and Smith, E. L., *Proc. Nat. Acad. Sci. USA* 69, 882 (1972).
12. Johns, E. W., *Biochem. J.* 92, 55 (1964).
13. Phillips, D. M. P. and Johns, E. W., *Biochem. J.* 94, 127 (1965).
14. Edwards, P. A. and Shooter, K. V., *Biochem. J.* 114, 227 (1969).
15. D'Anna, J. A., Jr. and Isenberg, I., *Biochemistry* 12, 1035 (1973).
16. Kelly, R. I., *Biochem. Biophys. Res. Commun.* 54, 1588 (1973).
17. Skandrani, E., Mizon, J., Santiere, P. and Biserte, G., *Biochimie* 54, 1267 (1972).
18. Li, H. J., Wickett, R., Craig, A. M. and Isenberg, I., *Biopolymers* 11, 375 (1972).