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RIBBON SHAPED HISTONE H4 AGGREGATES

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SUMMARY

The self-association process of histone H4 molecules into large aggregates is highly cooperative at an ionic strength of 0.03. Carefully prepared aggregates at this ionic strength are ribbon-shaped and take the form of right-handed helices. These ribbons have a uniform width of 20.5nm. The overall diameter of the "cylinder" formed by the helix is ca. 19.5nm, the average pitch of the helices 20nm, and the mean contour length 720nm. The thickness of the ribbon is less than 3nm.

It is well established that chromatin consists of a nucleosome structure. After discovery of the nucleosome structure, great attention has been paid to arginine-rich histones H3 and H4 because these two histone species seem to play central roles in nucleosome (1-3). Therefore, knowledge of the structures of histones H3 and H4 will give us valuable information on the nucleosome structure.

Aggregation of argine-rich histones has long been known (4-6) and electron microscopic observations of aggregates have been reported previously by Sperling et al. (7-9). In this paper, we tried to form a crystalline structure of H4 aggregates in order to study the detailed structure of this histone species.

MATERIALS AND METHODS

Histone H4 was prepared from calf thymus by the method of Johns (10) and purified by repeated precipitation with acetone (11). The histone H4 was found to be pure by gel electrophoresis (12) and amino acid analysis in comparison with the result previously reported for histone H4 (13).

The sedimentation measurements were carried out at 60,000 rpm in a Hitachi UCA-1A type ultracentrifuge using a synthetic boundary cell.

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The stain for electron microscopy was an aqueous solution of potassium phosphotungstate (1%) or uranyl formate (1%). The stains were adjusted to pH 5.7 with strong NaOH or HCl. The specimens were observed in a Hitachi HU-11A electron microscope equipped with an anticotamination device. Electron micrographs were taken at a magnification of 50,000 using an accelerating voltage of 75kV.

RESULTS AND DISCUSSION

Self-association process of histone H4 molecules with ionic strength increase. At ionic strengths lower than 0.02, a single slow-moving boundary was observed in the ultracentrifuge, whose $s°_{20,w}$ was 1.0 S. At an ionic strength of 0.03, only two boundaries were observed (Fig. 1. Inset). The $s°_{20,w}$ of the slow-moving boundary was 1.0 S and the fast-moving boundary, whose $s°_{20,w}$ was more than 25 S, corresponded to self-aggregates of histone H4. This fast-moving component could coexist with the slow one only at this ionic strength. At ionic strengths higher than 0.03, only a single, fast-moving boundary was observed. Consequently, there seems to be a breakpoint in the ionic strength below which only the slow-moving component can exist and above which the fast-moving component is solely observed (Fig. 1).

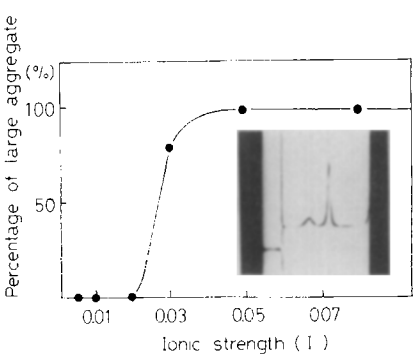

Fig. 1. Aggregation of histone H4 as a functions of ionic strength. The protein concentration was 4mg/ml and ionic strengths were adjusted with NaCl in 5mM Na-acetate buffer (pH 5.7). The inset shows the schlieren diagram of histone H4 (I=0.03). The photograph was taken 18 minutes after full speed was reached.

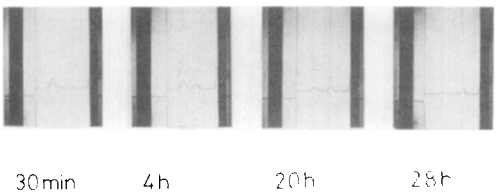

Fig. 2. Schlieren patterns of time dependent association of histone H4 molecules. Experiments were conducted with a protein concentration of 4mg/ml at 20°C (pH 5.7, I=0.03). The time written under the schlieren diagram indicates the hours after salt addition.

Time dependence of the self association. After the ionic strength increase of histone H4 in 5mM Na-acetate buffer (pH 5.7) up to the breakpoint (I=0.03), the transition from the slowly moving component into the fast-moving one was induced very slowly; the ratio of the slow-moving component in the schlieren diagram gradually decreased, while the ratio of fast-moving one slowly increased as a function of time (Fig. 2). At 30 minutes after the increase of ionic strength, the fast-moving one in the schlieren diagram were found to be 10%. While, at 28 hours, it reached its maximum of 80%. During this time-dependent change, only these two sedimenting boundaries could be observed. Such phenomena could not be seen at any other ionic strengths studied in our experiments.

Electron microscopy. The sedimentation experiments have revealed that the ionic strength of 0.03 is critical for the association process of histone H4, which seems highly cooperative and specific. Therefore, the aggregation of histone H4 was performed with stepwise dialysis against 5mM Na-acetate containing NaCl up to I=0.03 at 4°C, followed by gradual raise of temperature to 25°C without stirring. Finally, the ionic strength of the solution was adjusted to 0.055.

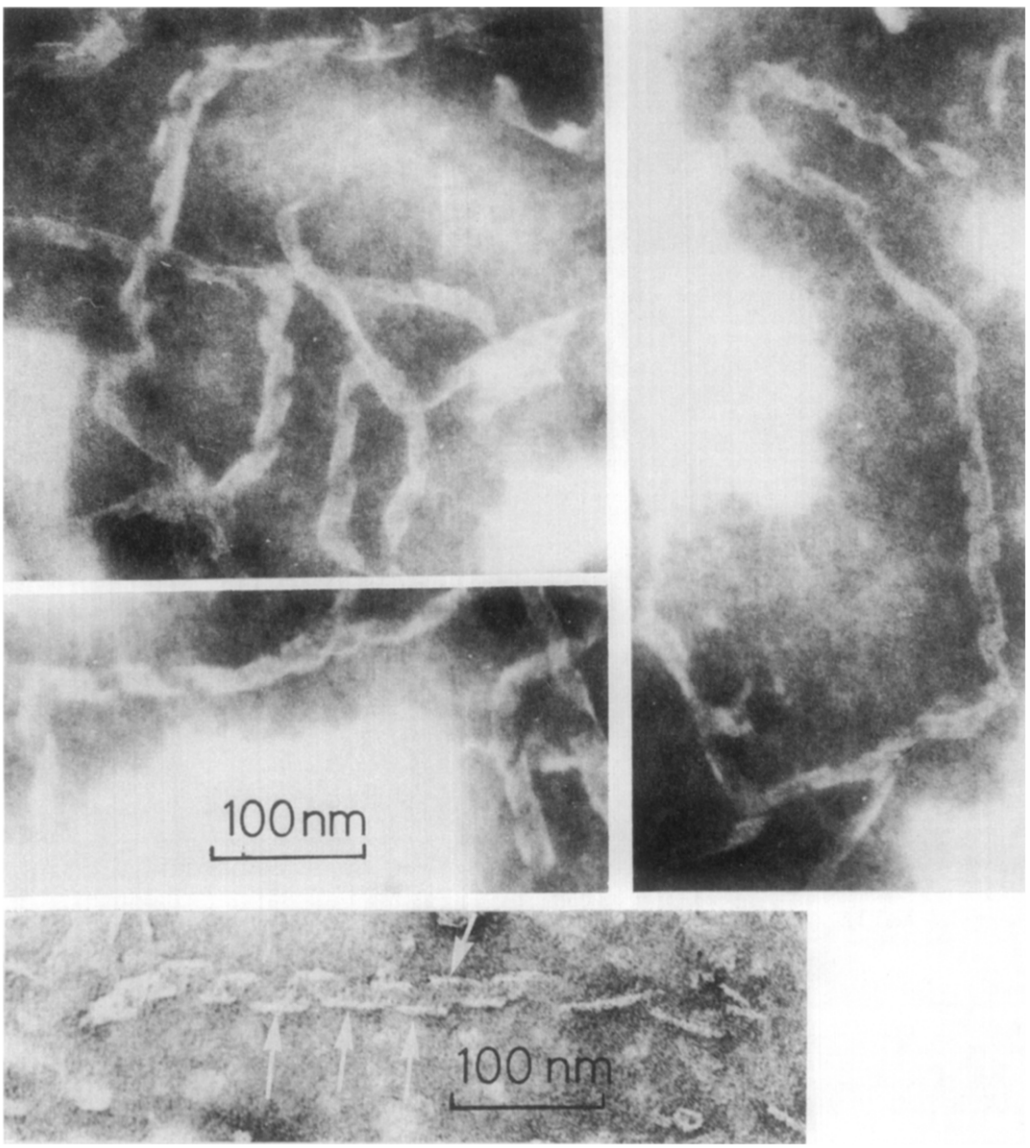

Fig. 3. Electron micrographs of histone H4 aggregates. The specimens were prepared at room temperature in 50mM NaCl- 5mM Na-acetate (pH 5.7).

Electron microscopic images of the H4 aggregates showed the shape of a helical ribbon (Fig. 3). The surface of the ribbon was almost in parallel with the central axis of the helix as if the tape were wound around a cylinder. The orientation of the helix was right-handed. The dimensions of the filaments were

determined from the electron micrographs. The width of the ribbon was found to be 20.5nm (S.D. 2.3nm) which was the mean of 135 measurements. Since the curvature of the ribbon made it difficult to measure the width exactly, the measurements are not necessarily accurate. Nevertheless, the calculated distribution of the width of the ribbon is so small that the width seems to be constant. The overall diameter of the "cylinder" was approximately 19.5nm (S.D. 4nm). The contour lengths of the ribbons differ from each other ranging from 200nm to 1,200nm. The mean length was about 720nm (S.D. 220nm). The pitches of the helices range from 40nm to 70nm and disordered occasionally. Although it was extremely difficult to evaluate the exact thickness of the ribbon, it was estimated by choosing appropriate images of micrographs. In negatively stained images, the fore and the rear faces of the ribbons are often superimposed and the ribbons appear translucent between two opaque "lines", which represent the sides (perpendicular to the supporting film) of the "cylinder" from that vantage point (Fig. 3. arrows). The approximate thickness of the ribbon can be estimated from these "lines" to be less than 3nm. Although the actual thickness of the ribbon must be thinner than this value since the curvature of two sides gives a wider image, this value should be close to the real thickness. Since the diameter of α-helix is about 1nm, the thickness of the ribbon may correspond to the diameter of the α-helix, or twice thereof.

It is well known that an amino acid sequence directs both secondary and tertiary structures of proteins and a number of attempts to predict such structures from amino acid sequences have been successful. We expect that the ribbon structure observed here will be useful not only for providing insight into the structure of histone H4 but also for elucidating the relation between amino acid sequence and protein structure.

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REFERENCES

1. Boseley, P.G., Bradbury, E.M., Butler-Browne, G.S., Carpenter, B.G. and Stephens, R.M. (1976) Eur. J. Biochem., 62, 21-31
2. Sollner-Webb, B., Camerini-Otero, R.D. and Felsenfeld, G. (1976) Cell, 9, 179-193
3. Moss, T., Stephens, R.M., Crane-Robinson, C. and Bradbury, E.M. (1977) Nucleic acids Res., 4, 2477-2484
4. Diggle, J.H. and Peacocke, A.R. (1971) FEBS Lett., 18, 138-140
5. Naito, J. and Sonnenbichler, J. (1972) Hoppe-Seyler's Z. Physiol. Chem., 353, 1228-1234
6. Ziccardi, R. and Schumaker, V. (1973) Biochemistry, 12, 3231-3235
7. Sperling, R. and Bustin, M. (1974) Proc. Natl. Acad. Sci. USA, 71, 4625-4629
8. Sperling, R. and Bustin, M. (1975) Biochemistry, 14, 3322-3331
9. Sperling, R. and Amos, L.A. (1977) Proc. Natl. Acad. Sci. USA, 74, 3772-3776
10. Johns, E.W. (1967) Biochem. J., 105, 611-614
11. Phyllips, D.M.P. and Johns, E.W. (1965) Biochem. J., 94, 127-130
12. Panyim, S. and Chalkley, R. (1969) Arch. Biochem. Biophys. 130, 337-346
13. DeLange, R.J., Fambrough, D.M., Smith, E.L. and Bonner, J. (1969) J. Biol. Chem., 244, 319-334