Histone octamer: dissociation in ultracentrifugal fields and subsequent reassociation into lower oligomers

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Tetramer, heterotypic *Core protein*

1. INTRODUCTION

An unsolved problem in our understanding of the nucleosome, the basic subunit of the eukaryotic chromatin (review [1]) is the manner in which histones H2A, H2B, H3 and H4, which form the core protein, are arranged among themselves. To approach this problem it is essential to obtain the core protein in a conformational state similar to that in intact chromatin. This condition appears to be achieved in 2 M NaCl at pH 7–9 [2–5]. However, there has been disagreement about the nature of the histone complex as it exists in 2 M NaCl:

(1) As proposed [3], the core protein in 2 M NaCl, as well as in the nucleosome core, is an octamer made up of two (H2A–H2B) dimers and an $(H3)_2$–$(H4)_2$ tetramer arranged as dimer–tetramer–dimer [6–9].
(2) The stable complex in 2 M NaCl is considered to be a heterotypic tetramer of one molecule each of H2A, H2B, H3 and H4 [10–12]; in [13] a weak association of heterotypic tetramers was found to form the octamer.

This disagreement about the nature of the histone complex in 2 M NaCl cannot be due to difference in source of histones because histone–histone interaction appear to be conserved during evolution despite differences in primary structure [14,15]. It appears rather that different conditions of preparation and analysis give different association–dissociation equilibria.

We showed in [16] that the core protein obtained gently and rapidly in 2 M NaCl at pH 7 is octameric with an $s^{\circ}_{20,w}$ of 6.6 S, a $D_{20,w}$ of 5.4×10^{-7} $cm^2 . s^{-1}$ and M_r 108000 ± 2500. This octameric complex undergoes a sharp conformational transition at centrifugal forces $\geq 60000 \times g$ to a species with an $s^{\circ}_{20,w}$ of 3.8 S, a value reported for the heterotypic tetramer [2].

Here, we show that the 3.8 S species in the centrifuge has an app. M_r much less than that of a tetramer but removal of the centrifugal field yields a species of histone having an app. M_r in the range assigned to the putative heterotypic tetramer.

2. MATERIALS AND METHODS

Histone H1 and the core protein were prepared as in [16,17]. Ultracentrifugal field relaxation experiments were done as in [18]. Sedimentation coefficients at 40000 rev./min were determined from synthetic boundary sedimentation velocity experiments [16]; diffusion coefficients were calculated as in [19]. Apparent M_r was calculated using Eisenberg's equation [20].

The nature of the molecular species formed by the dissociation of the histone octamer in the centrifuge was also examined by the sedimentation

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equilibrium method as follows: 0.11 ml of a dialysed sample of protein was taken in the solution sector of a synthetic boundary double-sector cell assembly. The diffusate (0.11 ml) was taken in the solvent sector. It was then centrifuged at 40000 rev./min for 1 h at 4°C. The rotor speed was then reduced to 20000 rev./min. After another 1 h the rotor-speed was further reduced to 10000 rev./min. The protein was allowed to attain sedimentation equilibrium at that speed at 10°C. Photographs were taken using interference optics until no more fringeshift occurred. After the run the protein solution was mixed by gentle shaking and an additional 0.25 ml of the diffusate was added to the solvent sector and the run was resumed at 8000 rev./min. After boundary formation photographs were taken at 4 min intervals using interference optics. From these photographs the total protein concentration in fringes was determined. The concentration of protein, C, as a function of radial position, r, was computed as usual [21]. Apparent M_r was calculated from a plot of log C *vs* r^2, using the equation:

$$M_r = \frac{4.606\ RT.Slope}{(1 - \phi\varrho)\omega^2}$$

Where R is the gas constant, T, the absolute temperature, ϕ the apparent specific volume of the protein, ϱ, the solution density and ω, the angular velocity in radians/s.

The nature of the histone species formed by the reassociation of the dissociation products in the absence of a centrifugal field was examined as follows: The core protein was centrifuged at 40000 rev./min for 1 h at 4°C; it was then mixed by gentle shaking and allowed to reassociate at 4°C for 3 h. The app. M_r of the product was determined by Archibald's approach-to-equilibrium method [21] at 18000 rev./min and 4°C. Calculations were done at the cell bottom.

3. RESULTS AND DISCUSSION

The M_r of the histone species calculated from Schlieren relaxation pattern after 4 min at 24000 rev./min was 102000 showing that the histones were initially present predominantly as an octameric complex. But the M_r calculated from Schlieren relaxation pattern (fig. 1) after centrifugation at 24000 rev./min for 10 min was 84000 indicating dissociation of the octamer.

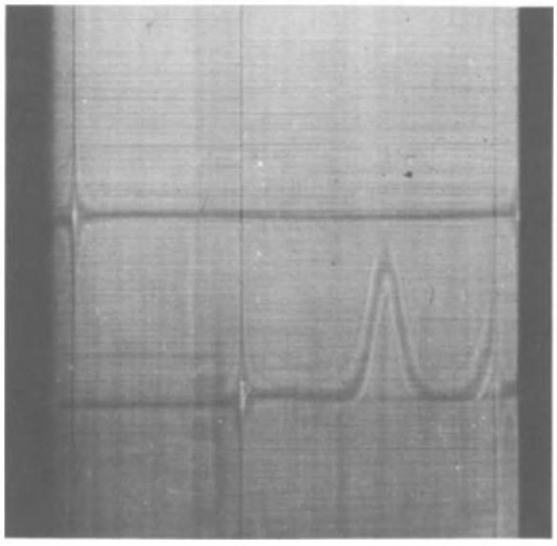

Fig. 1. Schlieren pattern of the ultracentrifugal field relaxation experiment on the core protein in 2 M NaCl. A Spinco model E analytical ultracentrifuge was used with the An-D rotor. The rotor temperature was maintained at 4°C. A scribed capillary type centrepiece was used in a 12 mm double sector cell. The protein was dialysed for 6 h at 0°C against the extraction buffer. The dialysed sample (0.12 ml, 6 mg protein/ml) was overlayered on fluorocarbon oil FC-43 (0.03 ml) in the solution sector. The diffusate (0.45 ml) was taken in the solvent sector. After boundary formation at 8000 rev./min the rotor was accelerated to 24000 rev./min and maintained at that speed for 10 min. The rotor was then decelerated to 10000 rev./min by coasting and photographs were taken at a bar angle of 60°C. Figure shows relaxation pattern after 34 min at 10000 rev./min.

At 40000 rev./min the core protein in 2 M NaCl at 4°C sedimented as one boundary [16] and the Van Holde plot of the sedimentation data (fig. 2) was linear. By these two criteria the histone species at 40000 rev./min appeared to be monodisperse [22]. The sedimentation coefficient observed under the experimental conditions was 1.6 S [16] which corresponds to an $s_{20,w}$ of 3.8 S. The sedimentation coefficient is the same as that of the putative heterotypic tetramer [2]. The diffusion coefficient in 2 M NaCl at 4°C obtained from the intercept of fig. 2 was 5.8×10^{-7} cm^2.s^{-1}. The app. M_r calculated by substituting the observed sedimentation coefficient

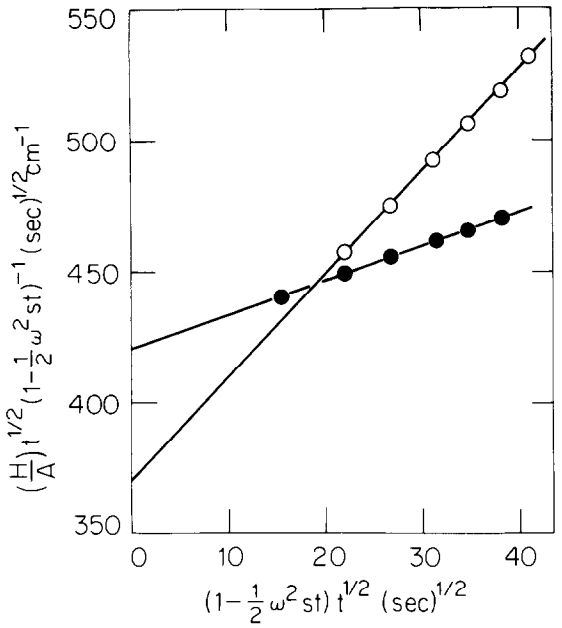

Fig. 2. Van Holde plots of synthetic boundary sedimentation velocity data at 40000 rev./min at 4°C on the core protein (○) in 2 M NaCl/25 mM sodium phosphate buffer (pH 7.0) and histone H1 (●,○) in 0.8 M NaCl/25 mM sodium phosphate buffer (pH 6.0). Each Schlieren picture was assumed to be an isoceles triangle and A/H was taken as equal to the width at half-maximal height.

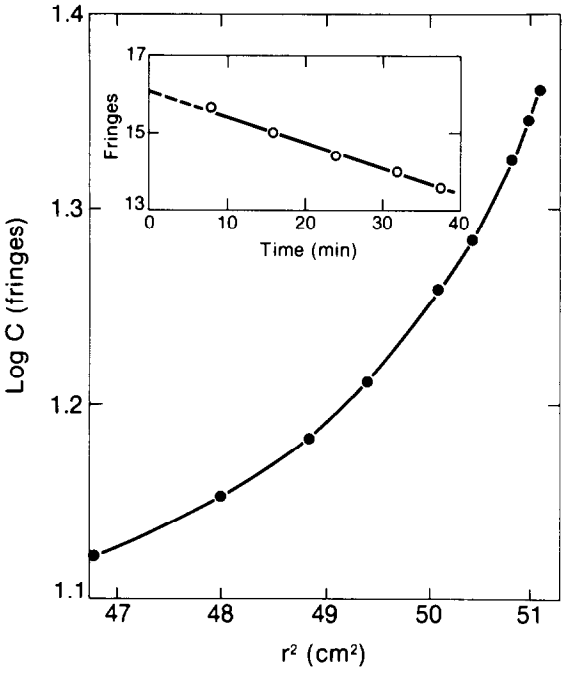

Fig. 3. Sedimentation equilibrium pattern of the histones on reducing rotor-speed to 10000 rev./min after centrifugation at 40000 rev./min for 1 h. Experimental details are given in the text. Photographs were taken with interference optics. Fringes were measured with the help of a microcomparator and the logarithm of the number of fringes was plotted against the square of the distance from the rotor centre. The inset is a plot of the number of fringes against time in a synthetic boundary experiment to determine total [protein].

and diffusion coefficient in the Eisenberg equation assuming an apparent specific volume of 0.73 ml/g [13,16] was 29000 which was very low for its sedimentation coefficient. Therefore a validation of the method used to calculate M_r was necessary and was done by using histone H1 in a similar experiment. A sedimentation coefficient of 1.0 S was obtained for this histone in 0.8 M NaCl/25 mM sodium phosphate buffer, pH 6.0 at 4°C. The diffusion coefficient obtained from the intercept of fig. 2 was 4.5×10^{-7} cm^2.s^{-1}. The M_r calculated by the above method using a partial specific volume of 0.74 ml/g [23] was 22000 which is in the range expected for histone H1 showing that the method cannot be faulted. Therefore the discrepancy between the sedimentation coefficient and the calculated app. M_r of the histones in 2 M NaCl at 40000 rev./min reflects a peculiar feature of the sedimentation behaviour of the histones at high centrifugal fields, not a limitation of the method. The basis for this behaviour, also observed at pH 9.0 [24], is not understood at present but might be related to the fact that the histones constitute an interacting system in the centrifuge. Certain rapidly equilibrating interacting systems might sediment as one boundary even though their constitutents might have different sedimentation coefficients [25]. When such systems are pressure-sensitive and when pressure favours dissociation, as in the case of the histones [16] potent convective instability of the concentration gradient could occur [26]. This may lead to spurious height–area relationships and M_r-values. But the estimated app. M_r does not seem to be far out as shown by the sedimentation equilibrium pattern (fig. 3). The

prominent curvature of the plot indicated marked heterogeneity. The app. M_r found at the bottom of the cell was merely 45000. That at the top of the cell was 14000, showing presence of monomeric histones. Clearly the starting material of M_r 102000 underwent extensive dissociation in the centrifuge but tetramers, heterotypic or otherwise, were not particularly prominent among the products. In fact, the app. M_r at the middle of the cell was ~23000 suggesting that the more prominent histone species might be dimers. It may be concluded that high-speed centrifugation of histone octamers results in their dissociation predominantly into dimers.

If the products of centrifugal dissociation of histone octamers were allowed to reassociate in the absence of any centrifugal field the app. M_r of the resulting material was found by Archibald's approach-to-equilibrium method, to be 60000. This turns out to be in the range of M_r-values reported for the putative heterotypic tetramer [2,10] but still far below that of the octameric starting material (M_r 102000).

Reassociation of subunits of oligomeric proteins is usually complete within a few minutes. The fact that reassociation of the products of dissociation of the histone octamer is not complete even after 3 h suggests that barriers to reassociation exist. The nature of this barrier could be discussed in terms [24]. Dissociation of the histone octamer by dilution in 2 M NaCl at pH 7.0 was found to proceed sequentially as:

$$(H2A)_2\text{–}(H2B)_2\text{–}(H3)_2\text{–}(H4)_2$$

$$\xrightarrow[\text{fast}]{(H2A\text{–}H2B)\ \nearrow} (H2A\text{–}H2B)\text{–}(H3)_2\text{–}(H4)_2$$

$$\xleftarrow[\text{slow}]{(H2A\text{–}H2B)\ \nearrow} (H3)_2 - (H4)_2$$

The dissociation was found to result in increased random coil structures. There is no compelling reason to believe that the pattern of dissociation in the centrifuge is different. In fact, the results of the ultracentrifugal field relaxation experiments and the M_r distribution in the sedimentation equilibrium experiment agree with this mode of dissociation. The tetramers appear to dissociate further under the influence of high centrifugal fields. It is reasonable to assume that the dissociation in the centrifuge is also accompanied by an increase in random coil structures. Thus the dissociation of the histone octamer results in an increase in the conformational entropy of the dissociation products of the order of 2–10 entropy units/mol random coil residues [27] in addition to the overall increase in entropy accompanying the dissociation process. The increase in the conformational entropy of the dissociated histones could be minimised if they could interact reversibly with an appropriate foreign substance in which case one can expect an increase in the rate of reassociation of the dissociated histones or in effect a slower rate of dissociation of the histone complex. This point of view affords an explanation for the puzzling observation first made in [28] and subsequently confirmed in [17] that histone complexes show higher app. M_r-values in Sephadex gels than in Biogels.

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