THE MOLECULAR WEIGHTS AND ASSOCIATION OF THE HISTONES OF CHICKEN ERYTHROCYTES

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1. Introduction

It now seems to be generally accepted that there are only a small number of well-defined histone fractions. Although considerable progress has been made in the chemical characterization of these fractions, there is relatively little knowledge of their physicochemical characteristics. Recent work [1] has shown that there are differences in molecular weights between the fractions, but so far no detailed work concerning the aggregation of histone fractions has been published. This short communication summarizes the results of our studies on the molecular weights and association behaviour of five of the main histone fractions (except F1) from chicken erythrocytes. Full details will be published later.

2. Materials and methods

The fraction F2c (nomenclature throughout is that of Butler et al. [2]) was prepared from chicken erythrocyte nucleoprotein by the method of Johns and Diggle [3]; the other fractions were prepared from the same source by methods similar to those described by Johns [4, 5].

The osmotic pressure (π) measurements were made at 25° in a Mechrolab 503 osmometer by the methods outlined previously [6]. The apparent number-average molecular weights (M_n^{app}) were obtained from the values of π/c at any given concentration (c g/l) and temperature.

Sedimentation equilibrium experiments were made with a Spinco model E ultracentrifuge. The 'white light fringe' method [7] was used to obtain the hinge point. The apparent weight-average molecular weights (M_w^{app}) were obtained from the slopes of the plots of $\ln J$ versus r^2, where J is the concentration in terms of fringes at r cm from the centre of rotation. The z-average molecular weight (M_z^{app}) was obtained from the slope of the graph of

$$\ln\left(\frac{1}{r}\frac{dc}{dr}\right)$$

against r^2.

The diffusion coefficient at infinite dilution (D°) of fraction F201 was determined by means of intensity fluctuation spectroscopy [8] and this yielded a molecular weight ($M_{s/D}$) after combination with its sedimentation coefficient at infinite dilution by means of the Svedberg equation. Partial specific volumes of the histone fractions were determined at 25° by means of an Anton Paar Digital Densimeter.

The concentration of the histone fractions were determined by the method described previously [6]; that is, the concentrations were obtained in terms of the weight of dry histone chloride per unit volume of solution so that the molecular weights obtained by both sedimentation equilibrium and by osmotic pressure measurements refer to one molecule of histone macromolecule and its associated chloride ions (following the procedures recommended by Carassa and Eisenberg [9]). The solvents for the histones were of ionic strength (μ) 0.1 and 0.5 which were made up of a 0.2 M constant ionic strength buffer (acetate, phosphate of glycine/NaCl, depending upon the pH required) and sufficient sodium chloride to bring μ to its required value.

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3. Results and discussion

Histone F2c was found to be very similar to F1 of thymus [10, 11]. The results obtained are summarized in table 1. The three weight-averages (M_w, M_z and M_n) can be seen to be in agreement and this in itself is good evidence for the mono dispersity of this fraction. Further evidence of monodispersity was afforded by the linearity of the ln J vs. r^2 plots at $\mu > 0.1$. At $\mu < 0.1$, these plots displayed the currature which results from non-ideality. The slopes of the $(M^{app})^{-1}$ vs. c plots increased with decreasing ionic strength. The plots of reciprocal apparent molecular weights (M_n^{app}, M_w^{app} and M_z^{app}) against c were all linear and it is the values so extrapolated to infinite dilution which are given in table 1. Histone F2c was not found to associate in any of the solvents employed (which varied from pH 4 to 7.5 and from $\mu = 0.1$ to 0.5). The molecular weight in 6M quanidinium chloride was 20,800 ± 500 which confirms the strictly non-associating character of this fraction.

Table 1
The molecular weight of histone F2c.

Solvent conditions				
pH	μ	M_n	M_w	M_z
7.5	0.5		22,300	
3.0	0.5		21,700	
4.0	0.5		22,500	
4.0	0.1		22,000	
4.0	0.05		21,200	
4.0	1.0		21,100	
7.5	0.1	21,900		
4.0	0.5	22,000		
4.0	0.1	22,100		
3.0	0.1	22,100		
4.0	0.5			23,100
6 M Gu Cl		20,800 ± 500	20,900 ± 50	

M_n from osmotic pressure measurements.
M_w and M_z from sedimentation equilibrium runs.

Histone F2b was found to associate with increasing ionic strength (table 2). The molecular weight (M_n) in 6M guanidinium chloride (14,400 ± 500) is in reasonable agreement with the value of 13,770 obtained from amino acid sequence work [12], after allowing for the contribution (of about 1200) of chloride ions to the former value. Aggregation occurred even at the lowest pH (2.0) and ionic strength (0.1), and increased with pH and ionic strength.

Histone F2a2 was found to be very similar to F2b, although the molecular weights obtained were much higher. The molecular weight (M_n) in 6 M guanidinium chloride was 16,800 ± 400, but the lowest M_n^{app} measured in the buffers was 25,000 (at pH 2, $\mu = 0.1$), which indicates a greater tendency to aggregate than with F2b.

Table 2
Molecular weights of histones F2b, F2a2, F3 and F2a1.

	Lys/Arg	M_n (at c=0) in 6M Gu HCl	M_w^{app}	M_n^{app}
F2b	2.5	14,400 ± 400	15,000 (μ = 0.1, pH 2) to 150,000 (μ = 0.5, pH 7.5)	15,000 (μ = 0.1, pH 2.0) to 60,000 (μ = 0.1, pH 7.5)
F2a2	1.1	16,800 ± 400	27,000 (μ = 0.1, pH 2) to 350,000 (μ = 0.5, pH 7.5)	25,000 (μ = 0.1, pH 2.0) to 200,000 (μ = 0.1 pH 7.5)
F3	0.8	19,100 ± 500	42,000 (μ = 0.1, pH 2) to 900,000 (μ = 0.1, pH 7.5)	40,000 (μ = 0.1, pH 2). At pH > 3, too large to measure
F2a1	0.9	13,000 ± 400	$M_{s/D}$ 2.9×10^6 (pH 2, μ = 0.1)	Too large to measure

M_n from osmotic pressure measurements.
M_w from sedimentation equilibrium runs.
$M_{s/D}$ by combining sedimentation and diffusion coefficients.

Histone F3 displayed an even greater tendency than F2b and F2a2 to aggregate with increasing μ and pH, and this rendered impossible any molecular weight studies by the methods available, at $\mu = 0.5$.

Histone F2a1 had a molecular weight (M_n) in 6 M guanidinium chloride of 13,000 ± 300 in agreement with the value of 11,280 from amino acid sequence work [13], again after adjustment (about 1100) is made for associated chloride ions. When this fraction was studied in the same solvents as used for the other fractions, it was found to be soluble at only $\mu = 0.1$. At pH > 2, and $\mu = 0.1$, a gel formed rather than a true solution so that studies could only be made at pH 2 and $\mu = 0.1$. Under these conditions it was found to have an s°_{25} of 25 S (compared with, for example, a value of 0.9 S for the $s^\circ_{20,w}$ for F1 [10]). The diffusion coefficient was, in fact, measurable at these concentrations (1.5 to 4 mg/ml) by intensity fluctuation spectroscopy [8] and was found to be 0.80 $(\pm 0.01) \times 10^{-7}$ cm^2 sec^{-1}. The linearity of the ln $[g^{(2)}(\tau) - 1]$ vs. τ plots [8, fig. 3] indicated that the species being studied was monodisperse. Combination of s° and D° by the Svedberg equation gave a molecular weight of 2.9×10^6, at pH 2, $\mu = 0.1$. Sedimentation equilibrium runs were also made at pH = 2 and $\mu = 0.1$ and the value of M_w so obtained was approx. 2.6×10^6 and again the material behaved, somewhat surprisingly, as if it were monodisperse.

From these results, the sequence for the tendency to aggregate of the fractions can be said to be:

$$2a1 \gg 3 > 2a2 > 2b > 2c \quad \text{(non-aggregating)}.$$

This observed sequence of aggregating tendency is in accord with that which has so far been postulated to explain the comparative broadening of certain parts of the NMR spectra of fractions 2a1, 2b and 2c [14, 15 and private communication from Drs. E.M. Bradbury and C. Crane-Robisnon]. The variations of M_w^{app} with c for histones F2b and F2a2 (reported only in outline in table 2) are ammenable to a more quantitative analysis, which is in progress. (In an earlier publication [6] we reported osmotic pressure, M_n, values for an acid-extracted "histone IIb". Further analytical work on this fraction showed it to be a mixture of F2c and F2a2, i.e., of a non-associating and an associating fraction, respectively, according to the later studies now reported. The variations in π/c with c then reported [6] are consistent with a simple summation of the contributions of the non-associating F2c and the self-associating F2a2.)

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References

[1] P.A. Edwards and K.V. Shooter, Biochem. J. 114 (1969) 227.
[2] J.A.V. Butler, E.W. Johns, D.M.P. Phillips, Progr. Biophys. Mol. Biol. 18 (1968) 211.
[3] E.W. Johns and J.H. Diggle, European J. Biochem. 11 (1969) 495.
[4] E.W. Johns, Biochem. J. 92 (1964) 55.
[5] E.W. Johns, Biochem. J. 105 (1967) 611.
[6] J.H. Diggle and A.R. Peacocke, FEBS Letters 1 (1968) 329.
[7] E.G. Richards and H.K. Schachman, J. Phys. Chem. 63 (1959) 1578.
[8] R. Foord, E. Jakeman, C.J. Oliver, E.R. Pike, R.J. Blagrove, E. Wood and A.R. Peacocke, Nature 227 (1970) 242.
[9] E.F. Casassa and H. Eisenberg, Advan. Protein Chem. 19 (1964) 28.
[10] A.J. Haydon and A.R. Peacocke, Biochem. J. 110 (1968) 243.
[11] D.L. Teller, J.M. Kinkade and R.D. Cole, Biochem. Biophys. Res. Commun. 20 (1965) 739.
[12] K. Iwai, K. Ishikawa and H. Hayaski, Nature 226 (1970) 1056.
[13] R.J. De Lange, D.M. Fambrough, E.L. Smith and J. Bonner, J. Biol. Chem. 243 (1968) 5906.
[14] M. Boublik, E.M. Bradbury and C. Crane-Robinson, European J. Biochem. 14 (1970) 486.
[15] M. Boublik, E.M. Bradbury, C. Crane Robinson and E.W. Johns, European J. Biochem. 17 (1970) 151.