THE MOLECULAR WEIGHT OF LYSINE-RICH HISTONE*

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The molecular weight of lysine-rich histone has been studied by a number of workers, and figures ranging from 8,000 to 18,000 have been reported (Davison et al., 1954; Phillips, 1962). The wide range of these figures is probably due to two factors. The first is the demonstrated tendency of histones to be degraded (enzymically) during some isolation procedures (Crampton et al., 1957; Phillips and Johns, 1959; Dounce and Umaña, 1962). The second factor is the very strong cationic charge of these proteins which would be expected to give rise to a charge effect but could be overcome by use of a high pH or salt concentration. Since the various workers referred to above used different solution conditions, the factors just mentioned could account for the variety of apparent molecular weights. Furthermore both factors tend to give apparent molecular weights which are too low, and so the higher values might well be the preferred ones. In spite of this, the value generally accepted for the molecular weight of this histone is about 10,000 (Murray, 1965).

In a reinvestigation of this matter, lysine-rich histone has been isolated in the most direct manner possible in order to minimize degradation, and then it has been submitted to ultracentrifugal analysis at high pH and salt concentrations. These experiments included tests which showed that the preparations used were essentially homogeneous with respect to molecular

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weight, and it was shown that the molecular weight determined was not distorted by a charge effect, or by the binding of salt. The molecular weight obtained, in conjunction with certain chemical data suggests that the recognized inhomogeneity of lysine-rich histone is a type of micro-heterogeneity.

Experimental

Lysine-rich histone was prepared as quickly as possible by the method of de Nooij and Westenbrink (1962) and was found to be free of degraded material as tested by polyacrylamide gel electrophoresis (Reid and Cole, 1964).

Ultracentrifugation was performed in a Spinco Model E ultracentrifuge, focused at the two-thirds plane of the cell (Svennson, 1956). A machine equipped with an electronic speed control was used for the sedimentation equilibrium experiments.

For the molecular weight determinations the Yphantis (1964) technique of high speed sedimentation equilibrium was used. The cell utilized for this work consisted of a six-channel Kel-F centerpiece with sapphire windows. FC-43 fluorocarbon from Minnesota Mining and Manufacturing Co. was used to provide a false bottom in each sector. In order to eliminate window distortion at high speed, a polaroid was inserted between the light source and lower collimating lens of the ultracentrifuge. Experiment A of Table I was performed at 36,000 r.p.m. while experiments B and C were performed at 40,000 r.p.m.

Photographic plates of both the equilibrium and solvent-solvent baseline experiments were read three to five times using a Gaertner microcomparator. Quarter or half fringe increments were read until that point in the cell at which the fringes were no longer resolved by the photographic plate. Above displacements of 16-18 fringes, the resolution of the photographic plates was insufficient to determine fringe positions.

The buffer used was either 0.50 or 0.25 M NaCl with 0.01 M NH_4Cl

at pH 9.2. The $\bar{V}$ calculated from the amino acid analysis by the method of McMeekin et al. (1949) was 0.76 cc/gm.

The calculations for these experiments were performed on an IBM 1620 Model II computer. The details of the computations will be presented at a later date (Teller, Richards, and Schachman, in preparation). Number and weight average molecular weights were calculated at every measured point in the cell. In addition, the approximation $M_1 \simeq 2 M_{n,x} - M_{w,x}$ (Yphantis, 1964) was calculated at each point and extrapolated to C = 0. Using the value of M_1, the following equation (Teller and Schachman, 1964) was calculated:

$$\frac{C_x}{L_x} = C_{p,1} + C_{p,2}L_x + C_{p,3}L_x^2 + \cdots$$

where $$L_x = \text{Exp}\left[\frac{(1-\bar{V}\rho)\omega^2}{2RT} M_1 (x^2 - x_p^2)\right]$$

x_p is the third x-position and $C_{p,i}$ the concentration of i-mer at that point. This allows a test of whether or not the system can be characterized by an association of a monomer unit to higher polymers. In addition, assuming the meniscus region is composed entirely of the protein with molecular weight M_1, the predicted curve of this small component was subtracted from the concentration at each point and the molecular weight of the remaining heavy material determined (Yphantis, 1964).

Results

The results of seven determinations of molecular weights and component analyses are summarized in Table I. Since M_1 (which cancels out virial coefficients) agrees with M_n and M_w (Yphantis, 1964) and since $M_n \leq M_w$ in general, it is clear that no charge effects occur at the high pH and salt concentration used. Table I (C-2) which shows a considerable fraction of heavier component is an artifact due to random error in plate reading and the nature of the computational methods used, as has been verified by statistical calculations with simulated mixtures. All the other figures indicate no statistically significant amount of dimer or

TABLE I

Summary of Sedimentation Equilibrium Experiments with Histone

Experiment	Cell	Approximate Initial Concentration (Per Cent)	M_n	M_w	$M_1^{\#}$ (C=0)	$\frac{M_w}{M_n}$	Per Cent Heavy Component	M_n of Heavy Component	M_w of Heavy Component	Per Cent Dimer	NaCl Concentration (Molar)
A	3	.09	21,100	21,200	21,000	1.0047	0.33	86,000	86,000	4.70	0.50
B	1	.025	21,500	21,500	21,700	1.0000	-1.94	--	--	0.00	0.50
B	2	.05	20,900	21,400	20,600	1.0239	2.76	50,000	50,000	4.60	0.50
B	3	.09	20,200	20,700	19,600	1.0248	3.79	44,200	44,200	4.82	0.50
C	1	.025	22,700	23,700	22,300	1.0441	-0.36	--	--	3.77	0.25
C	2	.05	24,600	24,400	21,000	0.9919	50.87*	25,700	26,600	30.00*	0.25
C	3	.09	21,800	21,800	21,700	1.0000	-0.14	--	--	0.22	0.25
Average	--	--	21,800	22,100	21,100	1.0128	0.75	--	--	3.02	--
± Standard Deviation			±1,400	±1,400	±1,200	±.0186	±2.11	--	--	±2.28	--

*Not included in averages.

#$M_1 = (2M_n - M_w)_{C=0}$

heavy material. Furthermore these same figures can be taken as showing a high degree of homogeneity.

Due to the large number of polar amino acids in the histone molecule, the possibility of salt binding causing an increase in molecular weight was investigated. A preparation of histone which had been dialyzed against distilled water containing a deionizing resin (e.g., Bio-Rad AG 501-X8) and then lyophilized was dissolved in the 0.5 M NaCl buffer. A synthetic boundary experiment and a sedimentation velocity experiment were performed, using Rayleigh Interference optics. The initial concentration from the synthetic boundary experiment obtained within twenty minutes from the beginning of the run was 12.80 $\pm$ 0.02 fringes. The value of the initial concentration computed from radial dilution (Schachman, 1959) using the boundary position as the half-concentration point in sedimentation velocity at 67,770 r.p.m. was 13.01 $\pm$ 0.08 fringes. Hence it is concluded that any effect due to the binding of salt is within the experimental error of the molecular weight determinations.

As pointed out above, this histone preparation appeared quite homogeneous with respect to molecular weight. Although subsequent chromatographic fractionation of the lysine-rich histone revealed four components in approximately equal amounts (Kinkade and Cole, in preparation), it can be shown by comparing the experimental results with calculations for simulated mixtures that the four components probably have nearly equal molecular weights.

Amino acid analyses for tyrosine and phenylalanine (Phillips, 1962), and determination (Phillips, 1963) of the acetyl residues presumed to be amino terminal in the lysine-rich histone, yielded about a half a mole of each per 10,000 gm. Using a molecular weight of 10,000, these data indicated fractional numbers of residues per mole of protein, and understandably this was interpreted as an expression of inhomogeneity in the preparation. In contrast, using a molecular weight of 21,000, the same data indicate one tyrosine, one phenylalanine, and 1.2 acetyl residues, per mole, and

suggest a degree of homogeneity with respect to primary structure.

Further indications of homogeneity in primary structure can be based on published studies of the tryptic digestion of lysine-rich histone (Murray, 1964). Amino acid analysis yields 30-35 moles lysine + arginine per 10,000 gms protein so that about 30-35 tryptic peptides would be expected of a single molecular species of molecular weight 10,000. The finding of 60-70 tryptic peptides (Murray, 1964) was therefore taken as indicating heterogeneity. However, when the molecular weight is recognized as 21,000, the number of peptides found is just what would be expected if this protein preparation were homogeneous.

These data taken together indicate that the various components known to occur in preparations of lysine-rich histone have very nearly the same primary structure and so might be thought of as a microheterogeneous system.

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REFERENCES

Crampton, C. F., Stein, W. H. and Moore, S. (1957). J. Biol. Chem. 225, 363.
Davison, P. F., James, D. W. F., Shooter, K. V. and Butler, J. A. V. (1954). Biochim. Biophys. Acta, 15, 415.
deNooij, E. H. and Westenbrink, H. G. K. (1962). Biochim. Biophys. Acta, 62, 608.
Dounce, A. L. and Umaña, R. (1962). Biochemistry, 1, 811.
McMeekin, T. L., Groves, M. L. and Hipp, N. J. (1949). J. Am. Chem. Soc., 71, 3298.
Murray, K., in "The Nucleohistones", eds. J. Bonner, and P. O. P. T'so. (1964). Holden-Day, Inc., San Francisco, p. 21.
Murray, K. (1965). Ann. Rev. Biochem., 34, 209.
Phillips, D. M. P. (1962). Progr. Biophys. Biophys. Chem., 12, 211.
Phillips, D. M. P. (1963). Biochem. J., 87, 258.
Phillips, D. M. P. and Johns, E. W. (1959). Biochem. J., 72, 538.
Reid, B. R. and Cole, R. D. (1964). Proc. Nat'l. Acad. Sci., 51, 1044.
Schachman, H. K. (1959). "Ultracentrifugation in Biochemistry", Academic Press, New York.
Svennson, H. (1956). Optica Acta, 3, 164.
Teller, D. C. and Schachman, H. K. (1964). Fed. Proc. 23 abs. 684.
Yphantis, D. A. (1964). Biochemistry, 3, 297.