HISTONE STOICHIOMETRY IN CHICKEN ERYTHROCYTE NUCLEI

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SUMMARY: In order to establish a quantitative relationship among the major histone components in chicken erythrocyte nuclei, an electrophoretic analysis was performed on purified histone fractions and on total histone extracts. The relative staining of the histones was calculated from quantitative densitometry of the amido black complexes. By comparison with the relative staining of the pure histone standards, we have calculated the molar ratios of the histones in the total histone extracts. The calculated molar ratios are not consistent with previous suggestions of pairs of all histones per repeating chromatin unit (the ν bodies).

There is much evidence that chromatin fibers consist of linear arrays of spheroid nucleoprotein particles (1-18), (denoted by us as ν bodies) (2). Several models have been proposed suggesting a specific molar stoichiometry of the individual histones in the repeating chromatin unit (the ν body) (2, 11, 16). To further the development of an adequate working model for the structure of chromatin, the relative molar ratios of the five major histone components in chicken erythrocyte nuclei were measured, and the results are reported in this paper.

METHODS: Chicken erythrocyte nuclei were isolated from the blood of young adult white Leghorn chickens as described previously (2, 19). Total histones were prepared from isolated nuclei by extraction with 0.25 M H_2SO_4, or with 5 M urea, 2 M NaCl as previously described (20). Pellets of the histones were dissolved in 0.01 N HCl, dialyzed exhaustively against 0.01 N acetic acid, lyophilized, and dissolved to a concentration of 1 mg/ml in 0.01 N HCl for gel electrophoresis. Individual histone fractions were prepared according to the method of Sanders and McCarty (21), and were observed to be electrophoretically pure. The purified histone fractions were stored frozen in water. Aliquots were removed for

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amino acid analysis, and the remaining samples were quantitatively diluted in 0.9 N acetic acid containing 5% sucrose and 0.1% methyl green, for gel electrophoresis. Amino acid analyses were performed on a Beckman 120 C Amino Acid Analyzer (Beckman Instruments, Inc., Fullerton, California). From these analyses, the lysine content of each histone was determined. They were as follows: F2A1, 0.626 (μmol lysine/ml); F3, 0.327; F2A2, 0.779; F2B, 0.734; F1, 0.572; and F2C, 4.348. Using previously published data for the number of lysine residues per individual histone molecule, the molar concentrations of histone in the undiluted samples were calculated. Values for the number of lysine residues per histone molecule are as follows: F2A1, 11 (22); F3, 13 (23); F2A2, 14 (24); F2B, 20 (25); F1, 61 (26); and F2C, 51, assuming 24.1 moles % lysine and 212 amino acids per histone molecule (27).

Total histone extracts were analyzed on 15% acrylamide gels (0.5 X 24 cm), as described by Panyim and Chalkley (28, 29). Pre-electrophoresis was carried out at 100 volts overnight, and electrophoresis was performed at 200 volts for 16 hr. Purified histone fractions were analyzed on acid urea gels (0.5 X 8 cm), which were pre-electrophoresed overnight at 33 volts and electrophoresed at 100 volts for 4-6 hr. Gels were stained overnight in 0.1% amido black containing 20% ethanol and 7% acetic acid, and were destained by diffusion until the backgrounds were slightly blue. The gels were then removed from the destainer, placed in destaining solution containing several drops of 0.1% amido black, and allowed to equilibrate for several days until the backgrounds were uniform in color. Quantitative densitometric scans at 570 nm were recorded on a Gilford Model 2000 Spectrophotometer and analyzed as previously described (20).

All chemicals were reagent grade, and solutions were prepared with glass-distilled water.

RESULTS AND DISCUSSION: Total histone extracts and purified histone fractions were analyzed by gel electrophoresis, and the results of

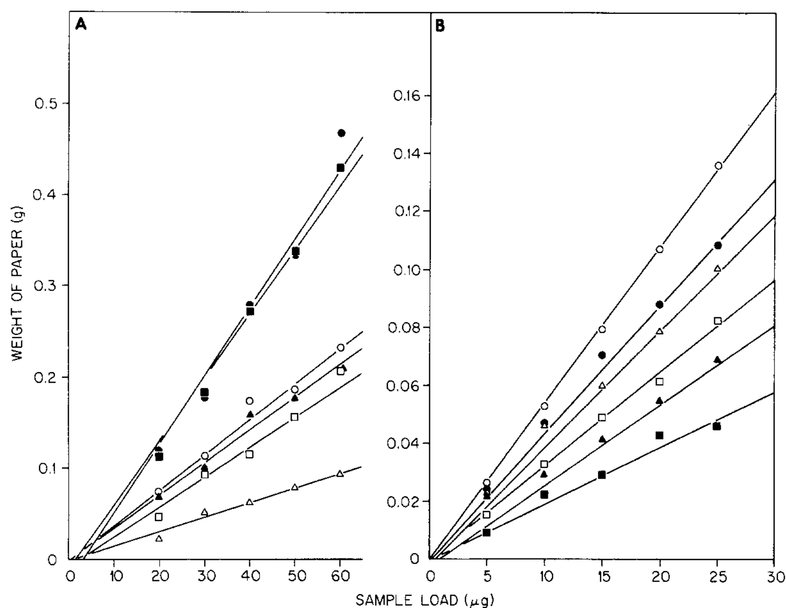

Figure 1: The results of quantitative densitometry on stained electrophoretic gels of total acid-extracted chicken erythrocyte histones (A) and on the purified chicken erythrocyte histones (B). The amount of stain absorbed at 570 nm by a histone peak is proportional to the weight of paper cut from a scan of that peak. The abscissa indicates the volume of sample loaded on the polyacrylamide gels prior to electrophoresis. Histones: F2A1 (□); F3 (O); F2A2 (▲); F2B (■); F1 (Δ); and F2C (●).

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quantitative densitometry are shown in Figure 1. These data indicate that there is a linear relationship between the area under each histone peak in the densitometric tracing (as measured by the paper weight) and the volume of sample loaded, thus confirming that: 1) Beer's Law is obeyed within the concentration range employed in this study and 2) the amount of stain absorbed per histone molecule remains constant as the quantity of histone is varied. From the slopes of least-squares lines fitted to the data of Figure 1A, the ratios of stain contained within the individual histone peaks (in total histone extracts) relative to the amount of stain absorbed by F2A1, were calculated; they are given in Table I (column 1). "Molar staining ratios" of the different histones were calculated from the slopes of a linear regression analysis of the data from Figure 1B, using the values of the molar concentrations of the

TABLE I

Relative Histone Content in Chicken Erythrocyte Nuclei

Histone	1 Relative Staining (Total Histones)	2 Molar Staining (Pure Histones)	3 Molar Ratio (Total Histones)
F2A1	1.00	1.00	1.00
F3	0.99	1.39	0.71 ± 0.06*
F2A2	1.05	0.86	1.22 ± 0.10
F2B	2.08	0.92	2.26 ± 0.20
F1	0.51	1.97	0.26 ± 0.02
F2C	2.08	2.70	0.77 ± 0.05

* Standard error values represent a minimum estimate, combining the standard errors of the slopes of the least-squares lines (Fig. 1A and 1B), neglecting errors in determining the histone concentrations and the moles of lysine per mole of histone. The total standard errors must be closer to ±20% of the calculated molar ratio.

pure histone fractions. The molar staining ratios are given relative to the staining of F2A1. As an example of the calculation of molar staining ratios, in one experiment the weight of paper from the F2C peak was 799.6 g/μmol of histone, that for F2A1 was 296.1 g/μmol, yielding a molar staining ratio of 799.6/296.1, or 2.70/1 (see Table I, column 2). The third column in Table I contains values for the molar ratios of histones extracted from chicken erythrocyte nuclei and were calculated by dividing the values in column 1 by the corresponding values in column 2. Total histones extracted from purified chicken erythrocyte nuclei by treatment with 5 M urea, 2 M NaCl yielded values of relative staining that were very similar to those of acid-extracted histones. The standard errors calculated for the molar ratios of histones (Table I, column 3) represent minimum estimates based upon the errors of the least-squares regression analysis. They do not include the errors in the determination of histone concentrations by quantitative amino acid analysis or errors

in the assumed values of the moles of lysine per mole of histone. We can only guess that these combined errors would be larger than those shown, perhaps as much as ± 20% of the calculated molar ratios. With such total standard errors, and assuming that the molar ratios of the histones might be integral values, the data would be consistent with the following molar ratios of individual histone fractions in the total histone extract: F2A1, 1.0; F3, 1.0; F2A2, 1.0; F2B, 2.0; F1+F2C, 1.0. Such integral values must be taken with considerable caution, however, since the total errors in the analysis could not be adequately measured.

Significant differences in the relative molar staining ratios of the five major histone components of chicken erythrocyte nuclei (Figure 1B, Table I, column 2) were observed. Fambrough et al. (30) observed that the extinction coefficients of the amido black-histone complexes (dye bound per unit weight of protein) from pea chromatin were independent of the type of histone. We have recalculated our molar staining ratios into relative staining per unit weight of histone (assuming the observed molecular weights for the different histone fractions) and do not find equivalent extinction coefficients. We do not know the basis for this discrepancy between our work and the previous study, but we would point out that the previous authors (30) used polychromatic light in measuring the dye-absorbances of histone complexes. Recently, McMaster-Kaye and Kaye (31) obtained spectra of all of the amido black-histone complexes and concluded that there were differences among these spectra. Additionally, they found that the relative amount of stain absorbed by individual histones is a function of destaining. In the quantitative study reported here, gels of total histone extracts were destained and scanned identically to those of the purified histone fractions. Therefore, we feel that any preferential enhancement of the staining of one histone fraction over that of the others, caused by the choice of 570 nm as the wavelength for

TABLE II

Molar Ratios of Histones in Different Tissues

		F2A1	F3	F2A2	F2B	F1	F2C
Mammal							
Calf	thymus	1.00	0.77	1.15	1.46	0.54	
Calf	intestine	1.00	0.87	1.27	1.41	0.52	
Calf	spleen	1.00	0.36	0.72	1.26	0.50	
Calf	lung	1.00	0.73	1.41	1.91	0.59	
Calf	brain	1.00	1.13	1.14	1.53	0.59	
Calf	liver	1.00	0.53	0.79	1.71	0.40	
Calf	endometrium	1.00	0.86	1.14	1.78	0.39	
Calf	kidney	1.00	0.83	0.71	1.53	0.50	
Dog	spleen	1.00	1.18	1.23	2.30	1.09	
Dog	thymus	1.00	0.98	1.43	2.00	1.16	
Dog	lung	1.00	0.78	0.74	1.90	0.35	
Opossum	lung	1.00	2.38	1.37	2.32	0.76	
Opossum	spleen	1.00	1.00	0.86	1.82	0.60	
Opossum	thymus	1.00	1.54	2.32	4.11	0.63	
Opossum	kidney	1.00	0.86	1.34	2.30	0.37	
Bird							
Hen	spleen	1.00	0.99	1.15	1.95	0.82	
Hen	blood	1.00	1.01	1.23	1.91	0.24	0.65
Hen	liver	1.00	1.01	1.19	1.92	0.80	
Hen	kidney	1.00	1.13	1.21	2.30	0.56	
Reptile							
Snapping turtle	kidney	1.00	1.99	3.21	4.07	1.47	
Snapping turtle	lung	1.00	0.50	0.74	1.33	0.25	
Snapping turtle	liver	1.00	2.08	2.49	5.51	1.49	
Snapping turtle	blood	1.00	1.19	1.97	2.65	0.89	
Amphibian							
Bullfrog	lung	1.00	1.12	1.37	1.96	0.86	
Bullfrog	intestine	1.00	1.00	1.18	1.83	0.64	
Bullfrog	liver	1.00	0.90	1.08	1.15	0.44	
Bullfrog	blood	1.00	0.90	1.02	1.42	0.76	
Fish							
Carp	intestine	1.00	0.65	0.96	1.53	0.31	
Carp	liver	1.00	0.88	1.05	1.32	0.55	
Carp	blood	1.00	0.98	0.82	1.41	0.33	

scanning, or any undue loss of one histone component due to over-destaining, in the whole histone extract, has been compensated for by the similar treatment of the histone standards.

Our data on the molar ratios of nuclear histone extracts suggest that there is no simple quantitative relationship among these histones

unless assumptions of the total standard error are made (see above). However, this fact is not obvious if one considers only the percent staining of each individual histone band (Table I, column 1). Previous data on the relative staining of hen blood histone extracts stained with amido black and scanned at 600 nm (32), are in good agreement with the data presented here (scanned at 570 nm). When these relative staining data are combined with our measurements of the molar staining ratios, calculated molar histone ratios are obtained (Table II). In an attempt to determine if any pattern arises from the histones of other sources, we have calculated molar ratios from the extensive data of Panyim and Chalkley (29) and Panyim _et al_. (32), by using our molar staining ratio data. We realize that error is inherent in such calculations since: 1) molar staining ratios of histones of different species may vary and 2) the molar staining ratios reported in Table I were obtained at 570 nm, while the data of Panyim and Chalkley (29) and Panyim _et al_. (32) were measured at 600 nm. It is clear from these calculations (Table II) that there is no simple relationship among the five major types of histone in various chromatin species. Such data are not consistent with our previous suggestion of pairs of F2A1, F3, F2A2, F2B and F1 (or F2C) per ν body (2), or the later suggestion of Kornberg of pairs of F2A1, F3, F2B, F2A2 and one F1 per repeating unit (16). These data (Tables I and II) suggest at least two possibilities of histone stoichiometry per ν body: 1) If all ν bodies of any tissue type are alike, the histone molar ratios are not simple equimolar relationships and may differ in different tissues. 2) Alternatively, not all ν bodies within a particular tissue type have identical histone stoichiometry.

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