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On the Similarity of Plant and Animal Histones*

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ABSTRACT: Histones prepared by acid extraction of purified chromatin from buds of pea seedlings and from calf thymus were fractionated by column chromatography on Amberlite CG-50, using a gradient of guanidinium chloride. Resulting histone fractions were further characterized by electrophoresis in polyacrylamide gel and by determination of amino acid composition and N-terminal amino acids. Striking similarities were noted between pea bud and calf thymus histones by every criterion of characterization employed.

Histones are basic proteins associated with deoxyribonucleic acid (DNA). They have been found in a wide variety of plant and animal phyla. Structural organization and stabilization of the genetic material (Zubay, 1964), regulation of DNA synthesis (Irvin *et al.*, 1963), and control of gene expression (Stedman and Stedman, 1950; Huang and Bonner, 1962) are among the many functions which have been suggested for histones. Although there have been numerous chemical studies of the histones of vertebrates (Murray, 1965; Phillips, 1962), histones of other organisms have been studied almost exclusively by histological methods (Das *et al.*, 1964; Bloch, 1962; Rasch and Woodward, 1959). Thus little is known about the properties of histones from organisms other than vertebrates. The fractionation and further characterization of pea bud histones, reported in this paper, make possible for the first time a comparison of the histones of very distantly related organisms. Such a comparison suggests answers to the questions: (a) Do the histones of all organisms have a common origin in evolution? (b) Do the histones of all organisms perform the same functions? (c) What chemical and physical properties of histones are essential to histone function?

Methods

Preparation of Chromatin. For the preparation of histones minimally contaminated by nonchromosomal protein it is necessary to use purified chromatin as the starting material for histone extraction. For the preparation of pea bud chromatin approximately 5 kg of pea seeds was soaked overnight in water, planted in vermiculite, and germinated in the dark for 4 days at 25°. The apical buds (approximately 1 cm of stem plus bud) were than harvested to yield about 600 g of fresh weight of buds. The buds were homogenized with approximately 1 l. of grinding medium (0.25 M sucrose–0.05 M Tris buffer, pH 8.0–0.001 M $MgCl_2$) for 1.5 min at 100 v in a Waring blender. This and all subsequent steps were performed at 0–5°. The homogenate was filtered through four layers of cheesecloth and then through two layers of Miracloth (Chicopee Manufg. Co., Miltown, N. J.). The filtrate was next centrifuged at 4000*g* for 30 min. The soft pellets were scraped from the underlying layers of starch, suspended in 300 ml of grinding medium, and centrifuged at 10,000·*g* for 20 min. The pellets were again separated from the starch, suspended in 300 ml of 0.05 M Tris buffer (pH 8.0), and centrifuged at 10,000*g* for 20 min. This step was twice repeated. The resulting pellets of crude chromatin were suspended in a total of 30 ml of 0.01 M Tris buffer (pH 8.0), homogenized with a Potter–Elvehjem homogenizer (about 20 strokes), and layered in 5-ml portions on 25-ml aliquots of 1.7 M sucrose in cellulose nitrate tubes. The upper two-thirds of the contents of each tube was stirred to form a rough gradient. The tubes were then centrifuged at 22,000 rpm for 3 hr in the SW-25 Spinco rotor. The resulting gelatinous pellets (purified chromatin) were suspended in 0.01 M Tris buffer and dialyzed against 100 volumes of the same buffer overnight. Recovery of DNA from the tissue homogenate was 70–80%.

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For the preparation of calf thymus chromatin the method of Marushige and Bonner (1966) was used without modification. This method is very similar to that described above.

Preparation of Histone. For extraction of histones the dialyzed chromatin was diluted to a concentration of less than 400 μg of DNA/ml with cold 0.01 M Tris buffer. This suspension was stirred on ice and one-fourth volume of cold 1 N sulfuric acid slowly added. After 30 min of stirring, the suspension was centrifuged at 17,000*g* for 20 min. The sediment was broken up and extracted with 0.4 N sulfuric acid (one-half the final volume of the first extract). These two extractions remove more than 95% of the acid-soluble protein. To the combined supernatants 4 volumes of cold absolute ethanol was added and the histone sulfate precipitated quantitatively at $-20°$ for 36 hr. The histone sulfate was recovered by centrifugation, washed three times with ethanol, and dried in a vacuum desiccator.

Column Chromatography and Recovery of Histones. Histones were fractionated by column chromatography on Amberlite CG-50, according to the method of Luck *et al.* (1958). Histones were dissolved in 8% guanidinium chloride, applied to a 2.5 × 55 cm column, and eluted with a linear gradient of buffered 8–13% guanidinium chloride (600-ml total volume) followed by 40% guanidinium chloride. All the guanidinium chloride solutions were buffered to pH 6.8 by using 0.1 M sodium phosphate. Fractions (4 ml) were collected. Protein content in the fractions was determined by turbidity (absorption at 400 mμ) after precipitation of protein from a 1.1 M TCA solution (Luck *et al.*, 1958; Bonner *et al.*, 1966). The remaining material from each histone peak was pooled and dialyzed against 20 volumes of cold 0.1 M acetic acid which was renewed at least four times. The volume of each dialysate was then reduced by rotary evaporation under reduced pressure at room temperature. The concentrated solutions were dialyzed extensively against 0.1 M acetic acid and then lyophilized. For the determination of chromatographic profiles on a purely analytical scale, a scaled-down fractionation procedure was developed (column 0.6 × 55 cm).

Electrophoresis. Disk electrophoresis was performed, using a modification (Bonner *et al.*, 1966) of the method of Reisfeld *et al.* (1962). A pH 4.3 gel which was 15% in acrylamide and 6 M in urea was prepared by mixing 1 volume of Temed solution (48 ml of 1 N KOH, 17.2 ml of glacial acetic acid, 4 ml of *N,N,N′,N′*-tetramethylethylenediamine, deionized water to 100 ml), 2 volumes of acrylamide solution (60 g of acrylamide, 0.4 g of *N,N′*-methylenebisacrylamide, deionized water to 100 ml), and 5 volumes of 0.2% (w/v) ammonium persulfate in freshly deionized 10 M aqueous urea solution.

Aliquots (0.9 ml) were pipetted into 6.5-cm lengths of 5-mm i.d. glass tubing and overlaid with 0.1 ml of 3 M urea for anaerobic polymerization. Each histone sample was dissolved at a concentration of 1 mg/ml in 10 M urea and 1–20 μl applied to a gel. This solution was overlaid with tray buffer (31.2 g of β-alanine, 8 ml of acetic acid, water to 1 l.) and electrophoresed in a standard disk electrophoresis apparatus at constant current of 4 ma/tube for 1.5 hr. Gels were stained for at least 4 hr in 1% amidoschwarz 10b–40% ethanol–7% acetic acid aqueous solution. The gels were then destained by electrophoresis and stored in 40% ethanol containing 7% acetic acid. The ethanol in the staining and storage solutions prevents swelling of the gels. Gels were scanned using a Canalco Model E microdensitometer (Canal Industrial Corp., Rockville, Md.).

Amino Acid Analyses. Histone samples (2–3 mg) were hydrolyzed in 2 ml of constant boiling HCl in evacuated, sealed tubes for 22 hr at 105°. Amino acid analyses were performed using a Beckman/Spinco automatic amino acid analyzer.

N-Terminal Analyses. N-Terminal amino acids of histone fractions were determined by a modified three-cycle Edman procedure (Edman, 1960; L. Hood and W. R. Gray, personal communication). Each histone fraction was dried *in vacuo* over phosphorus pentoxide and weighed immediately. Histone (2–5 mg) was suspended in 1 ml of coupling buffer (15 ml of pyridine, 10 ml of water, 1.18 ml of dimethylallylamine; this mixture was titrated to pH 9.0 with approximately 2 ml of 20% trifluoroacetic acid). To the suspension 50 μl of redistilled phenyl isothiocyanate was added, the suspension was flushed with nitrogen, and the coupling reaction allowed to proceed at 40° for 1 hr. The suspension was next washed with 5 ml of benzene and then four times with 4-ml portions of butyl acetate. Water (0.5 ml) was added to each tube and the mixture was lyophilized. The dried material was washed three times with 1-ml portions of ethyl acetate.

The phenylthiocarbamyl derivatives resulting from the above procedures were next cleaved by the addition of 0.2 ml of trifluoroacetic acid and incubation for 15 min at 40° under nitrogen. This cleavage and the subsequent cyclization produces 5-thiazolinone derivatives which were then extracted with successive 2-, 2-, and 1-ml portions of dichloroethane. The dichloroethane was removed by flushing with nitrogen. To the dried thiazolinones 0.3 ml of conversion buffer (30% ethanol titrated to pH 1.0 with 0.15 M HCl) was added, and the conversion reaction was carried out at 80° for 1 hr under nitrogen. The resulting 3-phenyl-2-thiohydantoin[1] amino acids were extracted with three 1-ml portions of ethyl acetate, the ethyl acetate was removed by flushing with nitrogen, and the PTH amino acids were dissolved in 50 μl of dichloroethane. For determination of yield 5 μl of the PTH amino acid solution was mixed with 1 ml of ethanol, its ultraviolet spectrum was determined, and the yield was calculated, using 16,000 and 14,300 for the molar extinction coefficients at 269 mμ of the alanine and proline derivatives (Edman and Sjöquist, 1956).

PTH amino acids were identified by thin layer chromatography on Eastman TSC fluorescent sheets, using two solvent systems: *m*-xylene and heptane–75% formic acid–dichloroethane (1:2:2) (systems D and F of Sjöquist (1960)). For system D the thin layer

[1] Abbreviation used: PTH, 3-phenyl-2-thiohydantion.

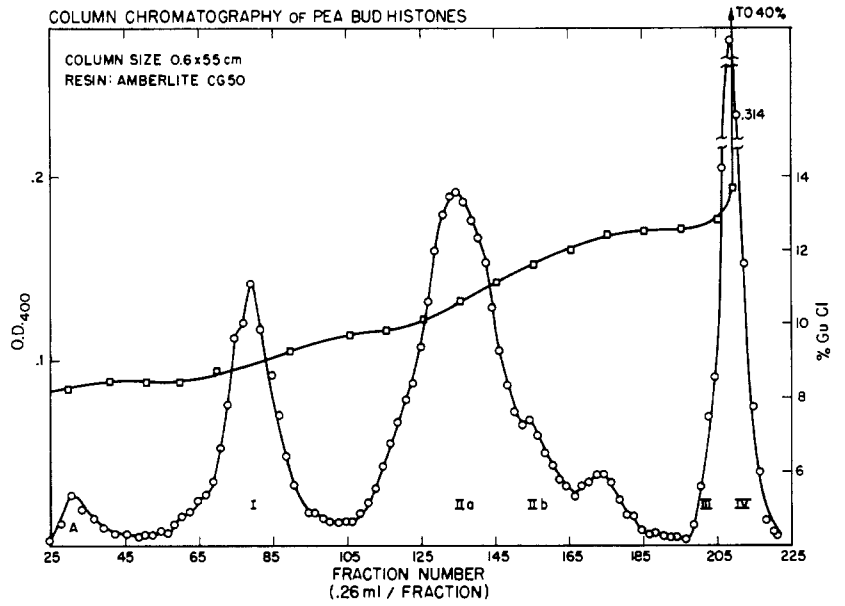

FIGURE 1: Fractionation of pea bud histones by column chromatography on Amberlite CG-50. Protein concentration in the effluent fractions was determined by optical density at 400 mμ of the turbid solutions resulting when the 0.26-ml fractions were mixed with 1.1 M TCA in a total volume of 1.56 ml (–O–O–). Concentration of guanidinium chloride in the effluent is indicated by □—□.

sheets were first treated with formamide–acetone (1:4) and dried briefly.

Identifications were confirmed and the existence of minor components was investigated by conversion of the PTH amino acids to dansylamino acids followed by electrophoresis in two buffer systems (Gray, 1966). The dansylation method is extremely sensitive and any dansylamino acid representing as much as 2% of the total dansyl derivatives is easily identifiable. To prepare dansylamino acids, 10 μl of the PTH amino acid–dichloroethane solution was combined with 30 μl of 0.1 N NaOH and hydrolyzed for 12 hr at 105° in a sealed tube. The pH of the hydrolysate was adjusted to about 8 by exposure to a CO_2 atmosphere. Dansyl chloride (60 μl) [1-dimethylaminonaphthalene-5-sulfonyl chloride (3 mg/ml) in acetone] was then added and the solution was incubated at 40° for 2 hr. The acetone was evaporated and the remaining solution was extracted twice with 60-μl portions of ethyl acetate (water saturated) and twice more at pH 4 (citrate buffer). The extracts were then dried and dissolved in 10 μl of 1 M ammonium hydroxide. Aliquots were spotted on paper and electrophoresed on a flat plate at pH 4.4 (pyridine–acetic acid–water, 10:20:2500, v/v), or at pH 12.6 (0.1 M trisodium phosphate–0.1 M sodium hydroxide). Electrophoreograms were inspected in ultraviolet light, the dansylamino acids appearing as strongly fluorescing spots at characteristic positions (some dansylamino acids such as dansylproline also have characteristic hues).

Results

Column Chromatography. Pea bud and calf thymus histones chromatographed on Amberlite CG-50 with a guanidinium chloride gradient yielded chromatographic patterns presented in Figures 1 and 2. Protein in the first peak (A) is not retained by the resin and appears immediately after one holdup volume of effluent. Three major histone fractions are separated by column chromatography. These fractions are labeled I, IIab, and III-IV to correspond to existing nomenclature for calf thymus histone fractions similarly separated (Rasmussen *et al.*, 1962). Amino acid analyses confirm that in both cases the three major fractions are, in order of elution, lysine rich, slightly lysine rich, and arginine rich. Calf thymus histone I is obviously heterogeneous, but its major subfractions have been shown to be of similar amino acid composition (Rasmussen *et al.*, 1962) and to possess virtually identical primary structures (D. M. Fambrough, unpublished observations; J. M. Kinkade and R. D. Cole, personal communication). Both pea bud and calf thymus histone II appear to consist of at least two chromatographic components, IIa and b, the predominant histone in pea bud being IIa, in calf thymus IIb. The small peak appearing after IIab in the pea bud histone pattern has been found by disk electrophoresis and by rechromatography to resemble unfractionated histone in its content of components I, IIab, and III-IV. Similarly, the calf thymus histone IIa resembles in electrophoretic behavior

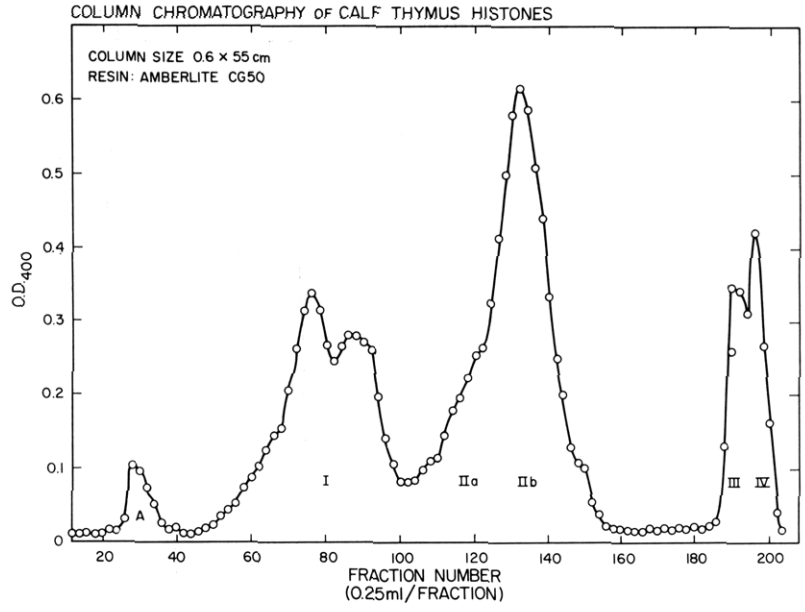

FIGURE 2: Fractionation of calf thymus histones by column chromatography on Amberlite CG-50.

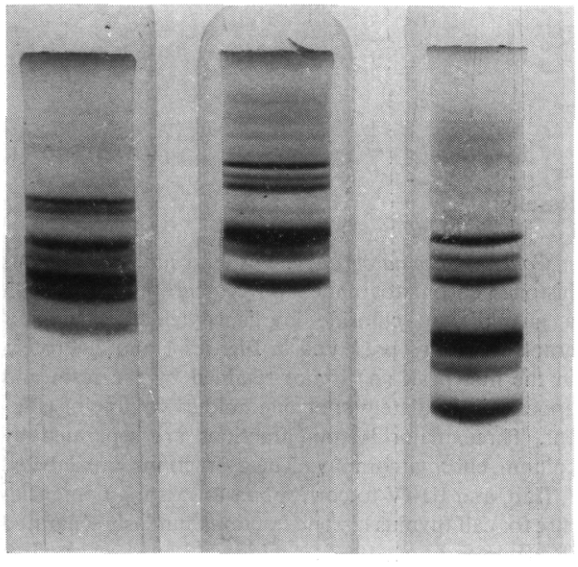

FIGURE 3: Fractionation of pea bud histones by disk electrophoresis in polyacrylamide gels. Electrophoretic migration was from top to bottom; gels were stained with amidoschwarz. Three different gel conditions are illustrated. From left to right they are: 15% gel, 15% gel containing 6 M urea, and 7.5% gel containing 6 M urea. A microdensitometer tracing of the center gel is shown in Figure 4.

and amino acid composition an aggregate of IIb and III-IV (see also Rasmussen *et al.*, 1962). The pea bud histone III-IV is sometimes partially resolved into two peaks which resemble calf thymus III and IV. Neither pea bud nor calf thymus fraction III-IV has been successfully fractionated into two components by the use of guanidinium chloride gradients of higher concentration. The subfractions III and IV have identical electrophoretic patterns and virtually identical amino acid compositions in both the pea bud and the calf thymus cases.

As is apparent from Figures 1 and 2 the relative amounts of the major histone fractions of pea bud and calf thymus histones are comparable. Since the amounts of histone extracted per milligram of DNA are also comparable (histone:DNA ratio = 1.14 for calf thymus, 1.31 for pea bud) it may be concluded that the *in vivo* histone compliment of pea bud and calf thymus are generally alike.

Disk Electrophoresis. Electrophoretic patterns of pea histones after electrophoresis in pH 4.3 polyacrylamide gels with and without urea are presented in Figure 3. Here the contribution of urea to resolution of the bands is evident, and it may be noted that only six major bands are observable and that the pattern is identical whether 7.5 or 15% gels are used. Densitometric tracings of amidoschwarz stained gels in which whole pea bud histone and histone fractions have been electrophoresed are shown in Figures 4 and 5. Pea bud histone I is composed of two major components plus a third component with mobility identical with that of IIa and which thus presumably represents some contamination by IIa. Histone IIa appears to be electrophoretically homogeneous except for a slight (1–2%) contamination by histone I material. Figure 5 shows the electrophoretic patterns of pea bud histone IIab, IIb, and a mixture of the two. Fraction III-IV (Figure 4) is composed of two major components of very different electrophoretic mobility plus some contamination by IIab. In the absence of urea much of the histone III-IV fraction fails to penetrate the gel. Several very minor bands of slower mobility are seen when fraction

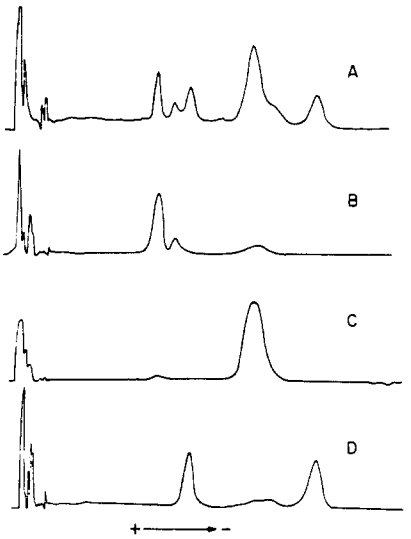

FIGURE 4: Microdensitometer tracings of polyacrylamide gels after electrophoretic fractionation of whole pea bud histone and pea bud histone fractions. Disk electrophoresis was performed in 15% polyacrylamide gels containing 6 M urea. Gels were stained with amidoschwarz and destained electrophoretically. The gels were then scanned using a Canalco Model E micordensitometer. Tracing A shows the electrophoretic fractionation of whole pea bud histone (see Figure 3). Tracings B–D show, respectively, the electrophoretic fractionation of histones I, IIa, and III-IV, all prepared by column chromatography on Amberlite CG-50. Peaks at far left indicate origin of gel, not stained material. The forward shoulder on the histone IIa peak in tracing A is histone IIb.

III-IV is electrophoresed, but these do not appear in the pattern of whole histone and must therefore represent artifacts of aggregation. The minor bands of low mobility observable in Figure 3 have been identified as contaminant proteins probably of ribosomal origin (D. M. Fambrough, unpublished observation).

Densitometric tracings of amidoschwarz stained gels in which calf thymus histone and histone fractions have been electrophoresed are shown in Figure 6. When applied in very low concentration histones Ia and b can be partially resolved. Whole Iab applied in larger amounts electrophoreses as a single component. Histone IIa has an electrophoretic pattern which resembles a mixture of IIb and III-IV and probably represents an aggregate of IIb and III-IV analogous to the aggregate found in the pea bud chromatographic profile (see also Rasmussen *et al.*, 1962). Histone IIb electrophoreses as a single major band. Histone III-IV consists of two major components, one with a mobility only slightly greater than that of IIb, the other of higher mobility. As in the case of pea bud III-IV, when calf thymus III-IV is electrophoresed, minor bands of lower mobility appear which are not found in the pattern of whole histone and thus must represent artifacts of aggregation.

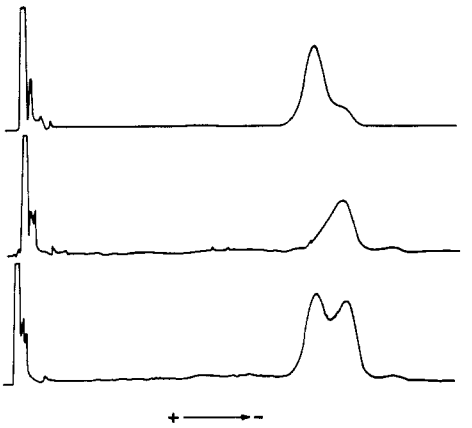

FIGURE 5: Microdensitometer tracings of polyacrylamide gels after electrophoretic fractionation of pea bud histone IIab (top), IIb (middle), and IIab plus IIb (bottom).

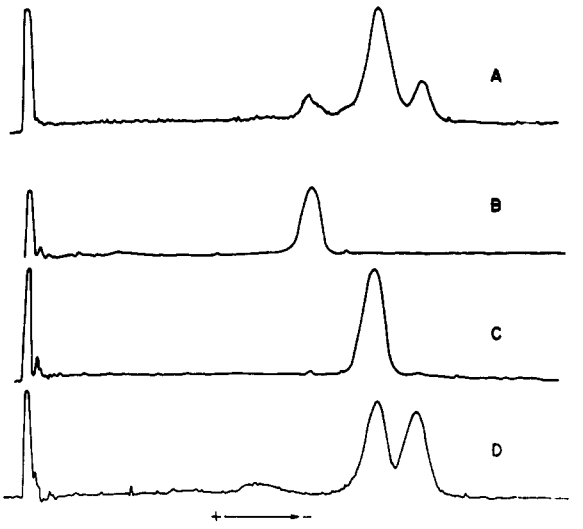

FIGURE 6: Microdensitometer tracings of polyacrylamide gels after electrophoretic fractionation of whole calf thymus histone (A) and calf thymus histone fractions Iab (B), IIb (C), and III-IV (D).

The similarities in the electrophoretic behavior of pea bud and calf thymus histones should be noted. The mobilities of pea bud and calf thymus histones are generally quite similar. In both cases also the lysine-rich histone components possess the lowest mobilities, the slightly lysine-rich components are of intermediate mobilities, and the arginine-rich fractions contain one component of highest mobility and another of intermediate mobility.

Amino Acid Analyses. The amino acid compositions of pea bud histone fractions are presented in Table I. These compositions are expressed as moles of the amino acid per 100 moles of total amino acids recovered. Although serine and threonine are partially degraded during hydrolysis, these losses are relatively small

TABLE I: The Amino Acid Compositions of Chromatographic Histone Fractions.

Amino Acid	Pea A	Pea I	Calf Thymus[a] Ib	Pea IIa	Pea IIb	Calf Thymus[a] IIb	Pea III–IV	Calf Thymus[a] III–IV
Lys	8.2	22.9	26.2	16.8	14.1	13.5	9.7	9.7
His	1.5	0.9	0.2	1.6	1.1	2.8	1.9	1.9
Arg	3.2	2.7	2.6	7.2	7.6	7.9	10.8	11.9
Asp	7.0	3.0	2.5	6.7	6.2	5.6	6.1	5.0
Thr	5.2	4.6	5.4	4.7	4.8	5.2	6.1	6.7
Ser	7.8	5.6	6.5	7.3	6.2	7.0	4.4	4.6
Glu	9.7	7.8	4.3	8.6	7.7	8.7	8.8	10.4
Pro	6.8	10.0	9.1	6.9	5.3	4.7	3.9	4.2
Gly	11.8	3.7	7.3	10.5	10.0	8.2	9.8	8.6
Ala	12.9	22.9	24.2	7.9	11.8	11.5	9.7	11.6
Val	7.3	6.2	4.1	4.5	6.9	6.7	6.7	5.9
Met	1.0	Trace	0.1	0.7	0.6	0.8	0.4	1.3
Ile	4.8	2.9	1.2	3.8	5.0	4.5	5.9	5.3
Leu	8.5	4.6	5.0	7.9	8.4	8.6	10.5	8.9
Tyr	2.3	0.9	0.7	2.4	1.8	3.0	2.4	2.2
Phe	2.2	1.3	0.6	2.7	2.4	1.3	3.1	2.5

[a] Taken from Rasmussen *et al.* (1962).

(approximately 10 and 5%, respectively; Rees, 1946), and no correction has been made for them. For comparison with the pea bud histone fractions, amino acid compositions of calf thymus histone fractions (taken from Rasmussen *et al.*, 1962) are also given in Table I. The calf thymus histone amino acid compositions are corrected for serine and threonine decomposition. The amino acid analyses were performed on histone fractions comparable to those, the electrophoretic patterns of which are given in Figures 4 and 5. Thus there is slight contamination of each fraction by material from other fractions. Moreover, the reported amino acid compositions are composites of the amino acid compositions of the electrophoretically separable components of each fraction. It is likely, however, that the electrophoretically separable components of each fraction have amino acid compositions quite similar to the composite composition. This is definitely the case for the lysine-rich components (Rasmussen *et al.*, 1962) and the arginine-rich components (Hnilica and Bess, 1965) of calf thymus histone fractions.

Comparison of amino acid compositions reveals striking similarities between corresponding pea bud and calf thymus histone fractions. Although caution should be used in comparative amino acid data of heterogeneous proteins, it may be concluded that all histones contain approximately 25–30% basic amino acids, principally lysine and arginine, and that they lack cysteine. Fluorometric analysis indicates that pea histones as well as calf thymus histones also lack tryptophan. The lysine-rich histones of pea bud and calf thymus show a most striking resemblance. Their arginine:lysine ratios are approximately 0.1. In addition to their high lysine content, these histones are equally rich in alanine, the two amino acids accounting for about half of the total compositions. Proline content is also remarkably high, about 9–10 mole %. Aromatic amino acids are present in very small amounts and methionine is lacking. The slightly lysine-rich histones of pea bud and calf thymus are generally similar. Of the two pea bud slightly lysine-rich histone components, IIb especially resembles the calf thymus slightly lysine-rich IIb, both with arginine:lysine ratios of about 0.5. There are only four amino acids present in amounts differing by greater than 1 mole %. The III-IV fractions are also extremely similar, differing in content by more than 1 mole % for only six amino acids.

N-Terminal Analyses. N-Terminal analyses of pea bud and calf thymus histone fractions are given in Table II. The N-terminal amino acids of the pea bud histone fractions seem to be identical with those of corresponding calf thymus fractions. The lysine-rich fraction yields no appreciable N-terminal derivatives (less than 1 mole/850,000 g of protein) and is presumably blocked by an acetyl group as, it also appears, is the calf thymus lysine-rich fraction (Phillips, 1963). Pea bud histone IIa yields predominantly proline as N-terminal group, with a much smaller amount of alanine. No other N-terminal groups are observed. The N-terminal group of the arginine-rich histones is exclusively alanine. The yields of N-terminal groups uncorrected for losses are 1 mole/59,000 g of protein for histone IIa and 1 mole/30,000 g of protein for histone III-IV. These values cannot be interpreted as molecular weights since all of the PTH derivatives may not be recovered. Previous estimates of molecular

weights based on recovery of N-terminal amino acids have been exceedingly variable (Phillips, 1962).

Discussion

Attention has been called to the many distinguishing characteristics of histones and histone fractions, but evaluation of the importance of each of these characteristics to the function or functions which histones perform cannot be made without detailed knowledge of the nature of the functions themselves. Until such knowledge is available the best criterion for the importance of the various histone characteristics is the constancy with which they are conserved in the histones of phylogenetically remote species.

TABLE II: N-Terminal Amino Acids of Pea Bud and Calf Thymus Histone Fractions.

Histone Fraction	Chief N-Terminal Amino Acids[a]	Ref
Pea bud I	Blocked	
Calf thymus Iab	Blocked	*b*
Calf thymus f1	Blocked	*b*
Pea bud II	Proline (major), alanine (minor)	
Calf thymus IIb	Proline 97%	*c*
Calf thymus f2b	Proline 78%, alanine 13%	*d*
Pea bud III-IV	Alanine (approx 100%)	
Calf thymus III	Alanine 74%	*c*
Calf thymus IV	Alanine 82%	*c*
Calf thymus f3	Alanine 95–99%	*e*

[a] Where proportions are given they are molar percentages of all N-terminal amino acids found. [b] Phillips (1963). [c] Satake *et al.* (1960). [d] Johns (1964). [e] Hnilica and Bess (1965).

The present comparison of pea bud and calf thymus histones suggests the importance of almost all of the previously noted histone features. Pea bud and calf thymus histones are both fractionated by ion-exchange chromatography into three major fractions: lysine-rich histone I, slightly lysine-rich histone II, and arginine-rich histone III-IV. The corresponding pea bud and calf thymus fractions are closely similar in amino acid compositions, have identical N-terminal groups, and display similar properties when electrophoresed in polyacrylamide gels. Very little heterogeneity is revealed by the techniques employed; column chromatography, disk electrophoresis, and N-terminal analyses reveal the presence of only six or seven major histone components in each case. If there is heterogeneity, it is likely to be confined to small sequence differences. It should be noted that disk electrophoresis carried out by the present method separates more than 20 species of ribosomal proteins. The complexity of pea ribosomal proteins has been previously demonstrated by starch gel electrophoresis (Setterfield *et al.*, 1960).

The striking similarities between pea bud and calf thymus histones suggest that a limited number of genes coding for these proteins originated early in evolutionary history and have maintained all of their major features since that time. It is improbable that such similarities could have arisen by independent convergent evolution. These similarities further suggest that the histones perform identical functions in a virtually identical manner in all creatures which contain histones. The constancy with which the distinguishing characteristics of histone fractions have been conserved through millions of years of independent evolution of plant and animal lines suggests that histones, unlike enzymes, possess no active site which is especially resistant to evolutionary change, but that many features throughout the primary structure of each type of histone molecule are important to its function.

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A Kinetic Study of the Reactions of Amino Acids and Peptides with Trinitrobenzenesulfonic Acid*

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ABSTRACT: The kinetics of the reaction of trinitrobenzenesulfonic acid with glycine, glycine peptides, and α-acetyllysine have been studied. The velocity constants are related to the pH of the reaction medium by the equation $\log k = a\text{pH} + b$. The values of a for glycine and α-acetyllysine are 0.92 and 1.0, respectively, and the value for both di- and triglycine is 0.56. The values of b for the first two compounds are −6.87 and −8.1, respectively, and the values for the last two are both −3.15. It is shown that trinitrophenylated (TNP) amino acids and peptides form complexes with sulfite ion giving association constants in the range of 10,000–21,000, corrected for the change with pH. The molar absorbance for the TNP-substituted ε-amino group is 1.15×10^4 and that for the α-amino group varies with chain length.

It was shown by Okuyama and Satake (1960) and Satake *et al.* (1960) that trinitrobenzenesulfonic acid[1] reacted with amino acids and peptides at relatively low temperatures. Since it was established that TNBS[1] reacted specifically with amino groups in a homogeneous aqueous system we have been studying the kinetics of the reaction of this compound with proteins. As part of this study we have investigated the reaction of TNBS with the model compounds glycine, di- and triglycine, and α-acetyllysine. All of the reactions followed a pseudo-first-order reaction when carried out in the presence of a large excess of TNBS. Evidence has been found that the trinitrophenylated (TNP) derivatives form a complex with sulfite ion.

Experimental Procedures[1]

Materials. Mono-, di-, and triglycine were obtained commercially and recrystallized from aqueous ethanol. α-Acetyllysine (*Anal.* Calcd: N, 14.9%. Found: N, 14.82%) was obtained from the Cyclo Chemical Co., Los Angeles, Calif., and on paper chromatography, showed a single component which differed from the ε-acetyl derivative and from lysine. The ε-acetyllysine was obtained from the same source (*Anal.* Calcd: N, 14.9%. Found: N, 14.8%); TNBS was obtained commercially and was recrystallized from 5 M HCl (mp 185°).

Trinitrophenyl derivatives of glycine (mp 162–163°), di- (mp 221°, dec) and triglycine (mp 235°), and α-acetyllysine (mp 119–220°) were made by the reaction of the amino acid or peptide with 100-fold molar excess of TNBS in 1% aqueous $NaHCO_3$ in the dark at room temperature for 24 hr. The products were recrystallized from aqueous methanol.

Methods. The reactions were carried out in stoppered 10-mm cuvets in a Bausch and Lomb 505 spectrophotometer connected to a Sargent recorder, Model SR. The absorbances were measured at 345 mμ unless otherwise stated.

Absorption spectra were obtained with a Bausch and Lomb 505 spectrophotometer and the absorbances were checked on a Beckman DU spectrophotometer. In all cases the agreement between these readings were within 0.005.

The reactions were run in 0.1 M sodium phosphate, 0.1 M sodium borate, and a mixture of 0.05 M phosphate plus 0.05 M borate buffers. All the studies were made at 16 ± 0.1°.

For the study of the interaction of sulfite with the trinitrophenyl derivatives, fresh solutions of Na_2SO_3 (chemically pure) were prepared in buffers and mixed with solutions of the pure trinitrophenyl derivatives after equilibrating both solutions at a temperature of 16°. The pH was determined with a Model G Beckman pH meter.

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[1] Abbreviations: TNBS, trinitrobenzenesulfonic acid; G, G_2, and G_3, glycine, glycylglycine, and triglycine; P, amino acid or peptide; ϵ_i, molar absorbance of compound "i;" SH^- and S^{2-}, bisulfite and sulfite ions.