A COMPARATIVE STUDY OF THE HISTONES OF SOME VARIETIES OF COTTON PLANT

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Tetraploid varieties of the cotton plant belonging to the medium- and thin-fibered types have been studied with respect to their histone/DNA ratio, their content of histone fractions, and the amino acid composition of the total histones. It has been shown that these varieties differ with respect to their contents of the lysine-rich histone fraction H1, and also by the amounts of certain amino acids in the total histone.

The comparative study of histones is of interest for elucidating the biological role of these proteins, which has not yet been definitively established. The broad competitive studies of the histones of higher eukaryotic organisms performed in the last decade have permitted the formulation of an idea of their separation into highly conservative (H3, H4), moderately conservative (H2a, H2b), and variable (H1, H5) fractions [1-3].

The comparative study of histones isolated from various vertebrates [4, 5] and from the sperm of various species of sea urchin [6] have shown that, in spite of their conservative nature, histones differ with respect to the amounts of definite fractions that are always present. This feature of the histones, characterized by a definite ratio between the individual fractions, is apparently biospecific [6].

We have performed a comparative study of the histones of different varieties of cotton plant. The histone/DNA ratio (Table 1), the amounts of various fractions (Table 2), and the amino acid compositions (Table 3) of different varieties of cotton plant were studied.

The histones were isolated from 2-day etiolated cotton-plant shoots by a modification of Alrey's method [7]. The amount of protein was determined by Lowry's method [8], and DNA spectrophotometrically [9] after separation by the Schmidt–Thannhauser method.

For the quantitative determination of each fraction of histones we used a method [10] consisting in the extraction of Amido Black 10B from the gels with acidified dimethyl sulfoxide and the determination of the optical densities of the extracts at 590 nm on a spectrophotometer. The method described gives a more accurate determination of the histones than ordinary scanning of the gel. This is connected with the fact that the different histones fractions stained by the dye in the polyacrylamide gel have their absorption maxima at different wavelengths, while the absorption maximum of the extracted dye is the same for all the fractions [10].

All the varieties of cotton plant that we studied were tetraploids belonging to two species (see Table 1).

In a comparison of figures for the histone/DNA ratio we see that this magnitude does not depend on the variety and species of cotton plant (Table 1).

The proportion of the arginine-rich histone fraction H4 was approximately the same in all the varieties (Table 2). The varieties of cotton plant differed in their contents of the labile lysine-rich fraction H1 (Table 2), there being a smaller amount of this fraction in the thin-fibered varieties than in the medium-fibered varieties.

The amino acid analyses of the total histones showed no appreciable differences between the varieties of cotton plants studied in the ratio of basic to acidic amino acids and the ratio of lysine to arginine. However, as can be seen from Table 3 the total histone of the

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TABLE 1. Histones/DNA Ratio in Different Varieties of Cotton Plant

Variety	Species	Ploidy	Histones/DNA*
108 F	Medium-fibered (G. hirsutum)	4n	1,08±0,07
Tadzhikistan	Medium-fibered) (G. hirsutum)	4n	1,03±0,05
Regar 1	Medium-fibered (G. hirsutum)	4n	1,05±0,05
5595 V	Thin-fibered (G. barbadense)	4n	1,04±0,07
Druzhba 60	Thin-fibered (G. barbadense)	4n	1,07±0,03
Druzhba 80	Thin-fibered (G. barbadense)	4n	1,02±0,08

*Each result was obtained from three experiments.

TABLE 2. Amounts of the H4 Fraction in the Sum of the Cortical Histones and of the H1 Fraction in the Total Histones (arithmetic mean values obtained in the analysis of three gels), %

Variety of cotton plant	H4*	H4†	H1*
108 F	22,8±0,2	21,0±0,6	23,4±0,2
Tadzhikistan	21,2±0,4	20,7±0,03	20,2±0,4
Regar 1	22,5±0,3	20,7±0,5	21,4±0,5
5595 V	22,4±0,3	20,3±0,7	17,1±0,7
Druzhba 60	22,6±0,4	22,3±0,5	16,2±0,4
Druzhba 80	22,6±0,3		16,6±0,6

*Determined according to [10] (method I).
†Determined by scanning gels stained by Coomassie R-250 (method II).

TABLE 3. Amino Acid Analyses of the Total Histones, mole %

Amino acid	Variety of cotton plant					
	108 F	Tadzhikistan	Regar 1	5595 V	Druzhba 60	Druzhba 80
Lysine	13,45	13,45	12,65	12,96	12,20	14,51
Histidine	2,14	1,99	1,67	1,84	1,38	1,41
Arginine	5,53	5,31	5,72	5,51	5,99	5,95
Aspartic acid	9,17	9,09	8,85	7,40	8,94	7,97
Threonine	4,53	4,59	4,73	5,36	4,41	5,13
Serine	7,74	7,56	7,32	7,33	7,61	7,43
Glutamic acid	12,14	11,57	12,62	12,67	13,66	12,30
Proline	5,69	6,03	6,40	9,53	6,93	6,24
Glycine	11,22	11,72	9,84	9,16	10,24	9,44
Alanine	11,34	12,63	11,56	12,03	11,60	12,51
Valine	3,18	3,05	3,53	2,10	3,59	3,62
Methionine	2,59	2,61	1,83	1,09	1,25	1,39
Isoleucine	1,19	1,21	2,01	2,35	1,72	1,95
Tyrosine	1,84	1,86	1,87	1,52	1,53	1,65
Leucine	5,80	5,59	6,76	7,11	6,60	6,58
Phenylalanine	2,39	2,16	2,42	1,95	2,36	1,91
Lys/Arg	2,43	2,53	2,21	2,35	2,03	2,44
Bases/acidic	0,99	1,00	0,93	1,01	0,87	1,08

thin-fibered varieties of cotton plant contained a larger amount of such amino acids as leucine, isoleucine, threonine, and glutamic acid and a smaller amount of aspartic acid, glycine, methionine, histidine, tyrosine, and phenylalanine, as compared with the medium-fibered varieties.

EXPERIMENTAL

Isolation of Nuclei and Histones from the Cotton Plant. The nuclei were isolated from 2-day etiolated cotton-plant shoots by a modification of Alfrey's method which we have described previously [7, 11]. The histones were isolated from the nuclei by extraction with 0.25 N HCl for 20 h.

Determination of the Amount of Histones in the Cotton-Plant Nuclei. After the extraction of the histones from the nuclei, the solution was centrifuged at 8000 rpm for 30 min, an aliquot was taken, and the amount of histones present was determined by Lowry's reaction [8], using calf thymus histone as standard.

Determination of DNA in the Cotton-Plant Nuclei. After purification, part of the nuclei was taken for determining the DNA content by the method of A. S. Orlov and E. I. Orlova [9].

The electrophoresis of the histones in long tubes was performed in 15% polyacrylamide gel as described by Panyim and Chalkley [12] at a urea concentration of 6.25 M. The dimensions of the tubes were 28 × 0.5 cm. Preliminary electrophoresis was performed for 18 h. The histones were dissolved in a buffer containing 4 M urea, 0.9 N CH_3COOH, 4% β-mercaptoethanol, and 0.2% pyronine, and 200 μg of histone was deposited on each tube. Electrophoresis was carried out at 200 V for 29 h. The gels were stained with Amido Black 10B and were eluted with 7% CH_3COOH.

Electrophoresis in a block of polyacrylamide gel was performed in a vertical block (150 × 130 × 1 mm) in the presence of sodium dodecyl sulfate by a modification of the method of Thomas and Kornberg [13] at a voltage of 60 V for 20 h. The total histone was deposited in an amount of 25 mg. The course of electrophoresis was monitored from the migration of Bromophenol Blue. After electrophoresis, the gel was fixed with 20% trichloroacetic acid for 30 min and was stained with a 0.1% solution of Coomassie Blue R-250. The gel was washed free from the dye by repeated treatment with 7% acetic acid.

Determination of the Percentage Amounts of the Histone Fractions. Method I. The bands stained by Amido Black 10B of the histones separated electrophoretically in fractions were cut out from the gels and the dye was extracted from the gels with dimethyl sulfoxide containing 1% HCl (1.2 ml for each 0.5 cm of gel) at room temperature for 20 h [10]. The optical densities of the solutions were measured at 590 nm on a Hitachi spectrophotometer. When determining the percentage amount of the H4 fraction, the optical density of all the core histones was taken as 100%.

Method II. One-millimeter blocks of polyacrylamide gels were scanned on an MF-2 microphotometer and curves were drawn through the points. The proportions of the individual fractions were determined after the densitogram has been transferred to Whatman paper and the well-separated peaks had been cut out and weighed.

Amino Acid Analysis. Samples were hydrolyzed with 6 N HCl in evacuated glass tubes at 105-115°C for 22 h. After the solvent had been evaporated off in a rotary evaporator and the residues had been kept in a desiccator over KOH, they were dissolved in citrate buffer, pH 2.2. The results of analysis were calculated in molar percentages and are given in Table 3.

SUMMARY

1. Tetraploid varieties of the cotton plant have been studied in relation to the histone/DNA ratio, the amounts of the various histone fractions, and the amino acid compositions of the total histones.

2. It has been shown that the cotton-plant varieties studied differ from one another with respect to their contents of the lysine-rich fraction H1.

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SYNTHESIS OF 3β-FLUORO DERIVATIVES OF 7-DEHYDROCHOLESTEROL AND OF ERGOSTEROL

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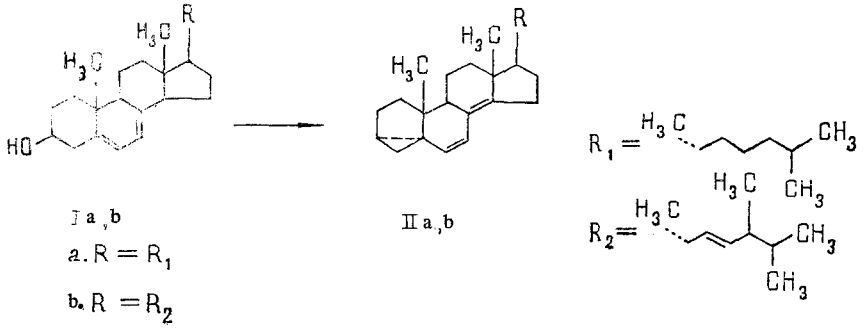
It has been established that the fluorination of 3β-hydroxy-$\Delta^{5,7}$-steroids, unlike that of 3β-hydroxy-Δ^{5}-steroids, does not lead to the formation of 3β-fluoro derivatives. The reaction products are 3α,5α-cyclo-$\Delta^{6,8(14)}$ compounds. Consequently, to obtain the 3β-fluoro derivatives of provitamins D — 7-dehydrocholesterol and ergosterol — the 5,7-diene system was first protected by the formation of a cycloadduct with 4-phenyl-1,2,4-triazoline-3,5-dione after which the adduct was fluorinated with morpholinosulfur trifluoride to the 3β-fluoro adduct, and then the 5,7-double bonds were regenerated by treating the adduct with a solution of sodium methanolate in methanol.

We have previously reported the synthesis of 3β-fluoro derivatives of vitamins D [1-3]. The main difficulty in their preparation is connected with the synthesis of the corresponding provitamins, since the direct fluorination of the vitamins and provitamins D does not form the 3β-fluoro derivatives. While the 3β-hydroxy group of cholesterol and of β-sitosterol is readily replaced by fluorine, in the fluorination of 7-dehydrocholesterol (Ia) and of ergosterol (Ib) with 2-choloro-1,1,2-trifluoroethylamine [4] or with morpholinosulfur trifluoride [5] the unsaturated hydrocarbon (II) were obtained with yields of about 90%. According to the results of elementary analysis, they had the empirical formulas $C_{27}H_{44}$ (for IIa) and $C_{28}H_{42}$ (for IIb), which was also confirmed by their mass spectra (presence of the molecular ions with m/z 366 and 378 and of fragment with m/z 253 [M^+ — 113 or 125 (side chain)].



Ia,b

a. R = R_1

b. R = R_2

IIa,b

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