HISTONES OF THE COTTON PLANT.

MOLECULAR CHARACTERISTICS OF HISTONE H1

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A characterization of the histones of two varieties of the cotton plant, Gossypium hirsutum and G. barbadense, has been given with the aid of various electrophoretic systems. Their molecular weights have been determined. An analysis has been made of the cleavage of histone H1 at tyrosine residues. The positive charge and the molecular length of histone H1 have been determined by the method of incomplete succinylation.

In contrast to the well characterized histones of animals, the main proteins of plant chromatin have been studied inadequantely. Investigations of plant histones have shown their similarity to animal histones [1], but at the same time there are differences. The most characteristic differences are the high molecular weights of the H2A, H2B, and H1 fractions of plant histones [2, 3]. The species specificity and subfractional composition of the H histones of triticale [4, 5], the peak [6], and maize [7, 8] have been studied.

We [9] have previously developed a scheme for the isolation and fractionation of cotton-plant histones. In the present paper we consider in detail the structural characteristics of the H1 fraction and the general characteristics of other histone fractions from two species of the cotton plant, Gossypium hirsutum (varieties 108-F and Regar-1) and G. barbadense (varieties 5595-V and Druzhba-60). By chemical cleavage at the tyrosine residues and by using the method of incomplete succinylation [11] we have determined the molecular weights and charges of the molecules of cotton-plant histones H1 and the number of tyrosine residues in it.

Figure 1 shows the electrophoretic separation of the total histone of the cotton plant performed in the presence of acetic acid–urea (Fig. 1, A) and of sodium dodecyl sulfate (NaDDS) (Fig. 1B). On comparing the patterns of electrophoretic separation of the histones isolated from the two varieties of cotton plant we were unable to detect differences between the materials studied. It is possible that analysis using longer gels could reveal differences in the subfractional composition of H1 like those that are known for the peak [6]. The H3 and H4 fractions of the cotton plant coincided in their electrophoretic mobilities with the corresponding proteins of higher animals. The H2A and H2B fractions of the cotton plant had lower mobilities than H3 in both electrophoetic systems and migrated as a single zone without separation. For the finer identification of the fractions we performed two-dimensional electrophoresis (in the first direction, electrophoresis by the method of Panyim and Chalkley, and in the second direction in the presence of NaDDS) (Fig. 1C). The results presented in Fig. 1 show that the H1 histones of the cotton plant consist of four subfractions which on separation by Laemmli's method migrated more slowly than histone H1 of calf thymus, which probably corresponds to a higher molecular weight of the cotton plant H1 histone.

One of the anomalous characteristics of plant histones must be considered to be their solubility in 5% perchloric acid, which is usually used for the selective extraction of the H1 histone. In an attempt to separate the H1 histone from the core histones of the total histone material of the cotton plant, 5% perchloric acid extracted, in addition to the H1 histone, other histones (mainly the H2A and H2B fractions), which made it difficult to obtain a pure preparation of H1 by the selective extraction method.

By using the molecular weights of the core histones of the calf thymus known from their primary structures for plotting a calibration curve, we determined the molecular weights of

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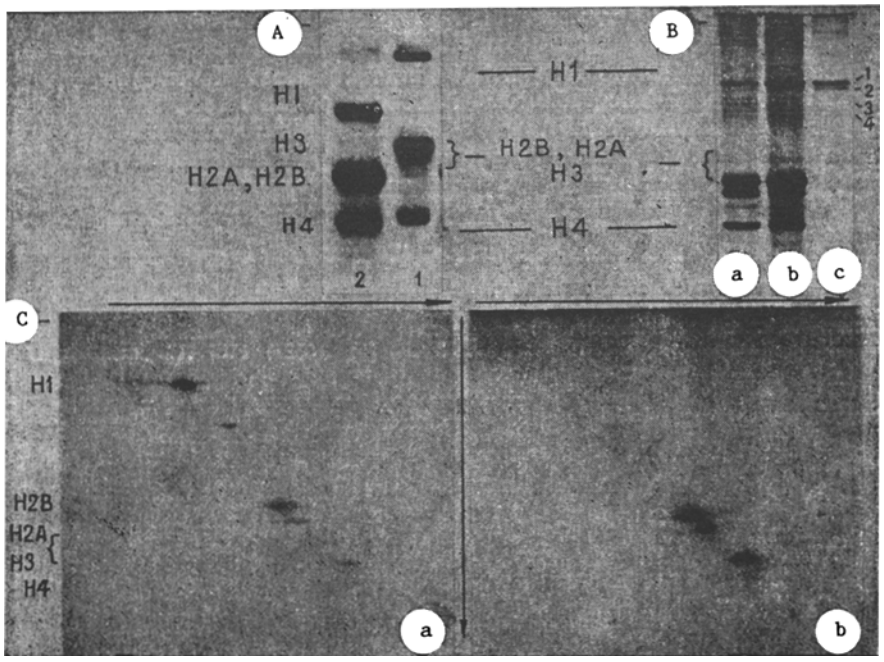

Fig. 1. Electrophoretograms of histones from cotton plant shoots. A. Electrophoretogram of the histones of a cotton plant of the species G. hirsutum (108-F) obtained by the method of Panyim and Chalkley (1) (the same pattern is observed for the histones from G. barbadense; not shown) in comparison with the histones of calf thymus (2). B. Electrophoretogram of cotton-plant histones by Laemmli's method: a) G. barbadense (5595 V); b) G. hirsutum (108-F); c) H1 fraction of a histone obtained by exclusion chromatography [9]. C. Two-Dimensional electrophoresis of the histones of a cotton plant of the species of G. hirsutum (Regar-1): 1st direction (left to right) by the method of Panyim and Chalkley; 2nd direction (from top to bottom) by Laemmli's method; 2) total histones; b) core histones.

TABLE 1. Molecular Weights (Daltons) of Cotton-Plant Histones Determined from the Results of Electrophoresis in the Presence of Sodium Dodecyl Sulfate*

Histone	Molecular weight
H2A	17800
H2B	
H3	15300
H4	11200
H1	28000

*The calibration curve for the core histones was plotted by using the molecular weights of the core histones of the calf thymus. The molecular weight of H1 was determined from a different curve plotted with the use of the molecular weights of sea urchin sperm H1 (27,000 daltons), H5 of chick erythrocytes (20,580 daltons), and H1 of the calf thymus (21,000 daltons).

the H2A, H2B, H3, and H4 fractions of the cotton plant from the experimental results on their electrophoretic mobilities in the presence of NaDDS (Table 1). It follows from Table 1 that the molecular weights of the H2A and H2B fractions of the cottom plant are substantially greater than the molecular weights of the corresponding fractions from higher animals. The determination of the molecular weight of histone H1 from the calibration curve plotted using the results for the core histones may lead to error, since the figures for H1

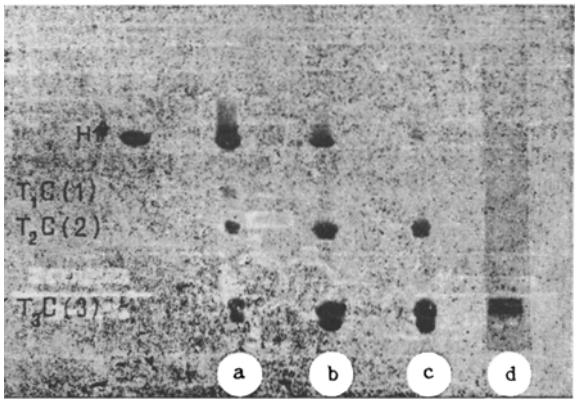

Fig. 2. Electrophoretogram of histone H1 of the cotton plant and its fragments obtained by cleavage with N-bromosuccinimide. Times of treatment of the protein with the N-bromosuccinimide solution (min): a) 3; b) 10; c) 15; d) 30. Time of separation 24 h; voltage 500 V, gel thickness 1.2 mm.

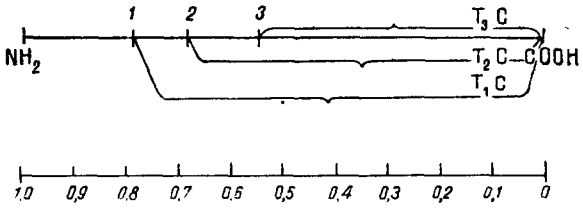

Fig. 3. Presumable positions of the tyrosine residues in the molecule of cotton-plant histone H.

do not fall on this curve. We therefore plotted a calibration curve on the basis of the known molecular wiehgts for histones H1 (from sea urchin sperm and calf thymus) and H5 of chicken erythrocytes and determined the molecular weight of the cotton plant H1 histones from the experimental results on their electrophoretic mobilities in the presence of sodium dodecyl sulfate in relation to the mobility of histone H4. The results of the analysis of the molecular weight values for the histones of the H1 family (see Table 1) show that the molecular weight of the cotton plant H1 histone is 28,000 daltons.

It is known that the H1 histone of higher animals is, as a rule, characterized by the presence of a single tyrosine residue located close to the globular segment of the molecule, while some subfractions of the H1 histone of salientians and sharks, and also histone H5 from chickens, have a large number of tyrosine residues [12]. The number and positions of the tyrosine residues may be criteria in the determination of the evolutionary closeness of the histones of the H1 family. To determine the number of tyrosine residues in the molecule of the cotton-plant H1 we performed a kinetic analysis of the products of the cleavage of this protein by N-bromosuccinimide (Fig. 2). On treatment for a short time (3 min) on an electrophoretogram, together with the intact H1, three fractions appeared corresponding to three fragments denoted by T_1C, T_2C, and T_3C, where T_iC is the polypeptide from the i-th tyrosine before the carboxy end of the molecule of histone H1. On prolonged separation (time 24 h, voltage 450 V, gel thickness 1.2 mm) of the cleavage products of histone H1 each of these fragments separated into two bands, which shows the presence in the main fraction of the H1 histone of a subfraction which is not separated under the conditions of its electrophoresis. The relative numbers of fragments were different, which probably corresponds to differences in the accessibility of the tyrosine residues to the action of N-bromosuccinimide. When the time of treatment with the cleaving agent was increased, all the material passed into fragment T_3C, possessing the highest mobility (Fig. 2). The tyrosine residue of fragment T_3C is probably most resistant to cleavage. When fragment T_2C was treated again with N-bromosuccinimide, fragment T_3C was formed, but this on further treatment with

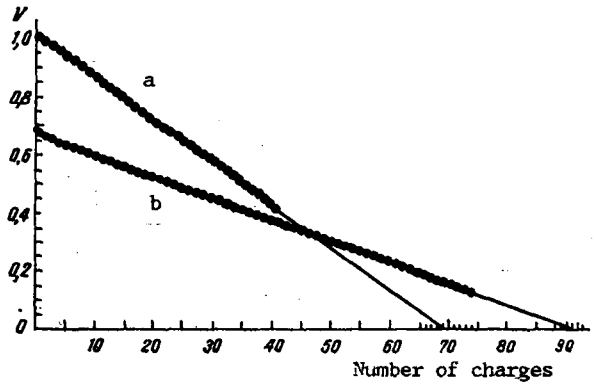

Fig. 4. Dependence of the electrophoretic mobility on the number of charges eliminated by succinylation for chick histone H5 (a) and for cotton-plant histone H1 (b).

TABLE 2. Some Molecular Characteristics of the H1 Fraction from Cotton-Plant Shoots Determined by Incomplete Succinylation

Protein	Number of lysine residues	Total positive charge	Number of Arg + His	Total number of amino acid residues
H5 of chick erythrocytes (our own results)	42	69	27	189
H5 from a knowledge of its primary structure [13]	42	69	27	189
H1 of cotton-plant	73	91	18	271

*Values for H5 given for comparison.

N-bromosuccinimide did not undergo any cleavage whatever and was the final product of the cleavage of the H1 molecule at tyrosine residues. The relative electrophoretic mobilities of the fragments T_3C, T_2C, and T_1C and of the uncleaved histone H1 were, respectively, 1.80, 1.44, 1.26, and 1.00. Thus, we may assume the following scheme of arrangement of the tyrosine residues in the cotton-plant H1 histone, as shown in Fig. 3, where the lengths of the fragments are expressed in arbitrary units of relative electrophoretic mobilities. According to the results shown in Fig. 2, H1 of cotton-plant shoots contains three tyrosine residues. The investigations described related to the main H1 fraction of the cotton-plant (see Fig. 1). Similar results were also obtained for the minor subfractions of the histone – 2, 3, and 4 –, the mobility of the T_iC fragment coinciding with the corresponding peptides of the main histone H1 fraction. This probably indicates that the differences between the subfractions of the cotton-plant H1 are connected with the N-terminal section of the molecule lying to the left of tyrosine 1 (see Fig. 3).

To determine the total positive charge and the molecular length of the cotton-plane histone H1 we used the method of incomplete succinylation [11]. Figure 4 shows the dependence of the electrophoretic mobility of the H1 fraction of the cotton-plant and the H5 of the chick on the number of positive charges "switched off" by succinylation after the treatment of samples with succinic anhydride. We find from the curves of Fig. 4 by extrapolation to the axis of abscissas that total positive charge of chick H5 is +69 (which corresponds to information on its primary structure [13]). The charge of the cotton-plant H1 histone is +91, and the number of lysine residues is 73 (see Table 2). The molecular length of cotton-plant H1 histone was determiend from formula given in the Experimental part. As a result it was found that histone H1 of the cotton-plant consists of 271 amino acid reisdues, which corresponds to an approximate molecular weight of 27,600 daltons. The value obtained for the molecular weight of histone H1 of the cotton-plane agrees well with the value of the molecular weight for this histone of 28,000 daltons determined from its electrophoretic mobility in the presence of NaDDS (Table 1). The molecular weight of the cotton-plant H1 is considerably higher than that from calf thymus (mol. wt. 21,000 [14]) and from maize (mol. wt. 24,000 [8]).

The present investigation has shown that the histones of the cotton-plant have features characteristic for the histones of other plants that have been studied – the existence of H2A and H2B with higher molecular weights that are specific for plants, the evolutionary conservatism of the H3 and H4 histones, and distinguishing features of the sequential organization of the H1 histone.

EXPERIMENTAL

The total histone was isolated from the nuclei of 2-day shoots by the method of Alfrey et al. [15], and histone H1 by fractionating the total histone on a column of Acrylex P-60, Reanal, as described in [9].

The proteins were analyzed with the aid of electrophoresis in a block of polyacrylamide gel in an acid medium by the method of Panyim and Chalkley [16] and in the presence of sodium dodecyl sulfate by Laemmli's method [17]. Two-dimensional electrophoresis was carried out as described previously [18].

Cleavage of the H1 Histone at the Tyrosine Residues. The corresponding protein zone was cut out from the gel used for the first direction that had been stained with Coomassie Blue R-250 according to [16] and was treated with a solution of N-bromosuccinimide (1 mg/ml) in 0.9 M acetic acid for the given time at room temperature. The treated lumps of gel were dried on filter paper and placed in a 4% solution of N,N,N',N'-tetramethylethylenediamine in 0.9 M acetic acid containing 8 M urea and 15% of sucrose for 30 min. Then the lumps of gel were placed in the pocket of a block of 20% polyacrylamide gel prepared by Panyim and Chalkley's method and previously subjected to electrophoresis. The electrophoresis and staining of the gels were carried out under standard conditions [16].

The incomplete succinylation of the H1 histone was performed as described by Rozov and Berdnikov [11].

In the experiment, the corresponding H1 band was cut out from the weakly stained (acetic acid–urea) gel and was steeped in a 3 M solution of sodium acetate for 1 h. Then the lumps of gel were transferred to a solution of succinic anhydride (2.5 mg/ml) in a mixture of dioxane and chloroform (2:1) for 15 min. After the treatment, the lumps of gel were placed in the packet of a 20% acetic acid–urea gel (length of separation 30 cm), and electrophoresis was carried out at a voltage of 20 V/cm for 48 h.

SUMMARY

1. The comparative characteristics of the histones of two species of the cotton plant, Gossypium hirsutum and G. barbadense have been studied.

2. The molecular weights of the cotton-plant histones have been determined from the results of electrophoresis in the presence of sodium dodecyl sulfate.

3. The number of tyrosine residues, the total positive charge, and the molecular length of the cotton-plant H1 histone have been determined.

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A STUDY OF THE ACID-BASE TRANSFORMATIONS OF OXODIHYDROTHIOCHROME

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The acid-base properties of oxodihydrothiochrome – the product of the redox disproportionation of the vitamin B_1 catabolite thiochrome (pH = 10.7, 4.5, and 0.5) – have been studied in the pH interval of 0-12 by PMR spectroscopy. It has been established that in acid medium (pH 0-1) the formation of a doubly charged ion is accompanied by a further structural transformation of the molecule of (I) with the formation of a vitamin B_1 analogue – oxothiamine (II). A possible mechanism of the action of (I) as an inhibitor of thiamine-dependent enzymes is discussed in the light of the results obtained. Details of the PMR spectra of (I) and (II) are given.

We have shown previously [1] that the natural catabolite of thiamine (vitamin B_1) thiochrome is converted as the result of redox disproportionation into oxodihydrothiochrome (I). The latter compound possesses a pronounced biological activity [2]; in particular, it inhibits transketolase – an enzyme catalysing the transfer of a glycolaldehyde fragment from a keto sugar to an aldo sugar. The coenzyme of this reaction is thiamine diphosphate. In view of the fact that the catalytic activity of thiamine and its analogues is a function of the pH of the medium, it appeared to us to be important to investigate the acid-base properties of oxodihydrothiochrome.

The study of the acid-base transformations was performed by PMR spectroscopy which not only enabled a quantitative estimate of them to be given but also revealed the corresponding sections of the molecule involved in these transformations. Analysis of the spectra (Fig. 1) showed that oxodihydrothiochrome undergoes three acid-base transformations in the pH range studied. As can be seen from Fig. 1, the first transition, in the alkaline region, has its greatest influence on the position of the 4-H signal ($\Delta\delta$ = 0.2 ppm), which indicates a nucleophilic attack of hydroxyl on the 5-C atom with the formation of the anion (II) (scheme). The upfield shift of this signal is due to a decrease in the screening effect of the hydrogen of the pyrimidine ring by the amide oxygen through a disturbance of the coplanarity between them and a change in the electric field as a consequence of the formation of a carboxylate ion.

The second transition in the weakly acid region is undoubtedly connected with the protonation of the cyclic secondary amino group. The formation of the ammonium ion (III) is reflected primarily in the position of the signal of the closest proton, 9a-H ($\Delta\delta$ = 0.13 ppm), and, to a smaller degree, in the position of the signals of the other proton-containing groupings directly connected with the pyrimidine and thiazole rings. The latter is due an increase in the "permeability" of the imine bridge for π-electrons of the two rings, which is clearly shown in the electronic spectra as a bathochromic shift of the long-wave absorption band ($\Delta\delta$ = 25 mm).

In more acidic media, the addition of a proton takes place at the most basic oxygen atom of the amide group with the formation of conjugate acids having the alternative structures (IV) and (V). The ambident nature of the cation (IV) finds its confirmation in the

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