ELECTROPHORETIC INVESTIGATION OF THE HISTONES OF THE COTTON PLANT IN POLYACRYLAMIDE GEL CONTAINING SODIUM DODECYLSULFATE

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In order to study the fractional compositions of the histones isolated from the cotton plant, they have been subjected to electrophoretic separation in polyacrylamide gels (PAAGs) containing sodium dodecylsulfate (NA-DDS). It has been established that the rate and sequence of migration of the histones under consideration in PAAGs differ from the corresponding values of the histones of the calf thumus. A comparative evaluation of the histones of the cotton plant and of the calf thymus has been made by two-dimensional electrophoresis. The molecular weights of fractions of the cotton-plant histone were determined from their electrophoretic mobilities in PAAGs with Na-DDS: H1 — 23,500; H2A — 14,600; H2B — 14,960; H3 — 14,300; and H4 — 11,300.

The comparative study of histones from various sources is of great interest for elucidating their functional role.

The present paper gives the results of an investigation of the histones from the cotton plant with the aid of disc electrophoresis in polyacrylamide gel (PAAG) in the presence of sodium dodecylsulfate (Na-DDS) in comparison with calf thymus histones.

We have previously [1] found certain differences between the histones with respect to electrophoretic mobility in an acid-urea gel [2], in amino acid composition, and in gel chromatography on Acrilex P-60. The cotton-plant histones are distinguished by a lower mobility in PAAG and earlier elution from a column than the calf thymus histones. Furthermore, there are differences in the sequence of elution of the fractions. For the cotton-plant histones — H1, H2B, H2A, H3, and H4; for the calf thymus histones — H1, H3, H2A, H2B, and H4.

For a more detailed study of the fractional composition of the cotton-plant histones we performed their electrophoretic separation in a block of gradient PAAG containing Na-DDS [3].

Figure 1 shows the results of the electrophoresis of the cotton-plant and calf-thymus histones after fractionation on an Acrilex P-60 [1]. As we see, the sequences of migration of the fractions of these histones in PAAG containing Na-DDS are different. In the first case (Fig. 1, I) the H3 fraction migrates beyond the arginine-rich H4 fraction, and in the second case (Fig. 1, II) beyond the H2A and H2B fractions. Furthermore, fractions H1, H2B, and H2A in the cotton-plant histones are less mobile than the corresponding fraction of the calf-thymus histones. The H4 histone proved to be the most conservative in this respect.

A comparative evaluation of the histone was also made by two-dimensional electrophoresis under identical conditions. The electrophoresis of the histones in the first (vertical) direction was carried out in acid-urea gel [2] (Fig. 2) and in the second (horizontal) direction in a block of PAAG containing Na-DDS [3] (Fig. 3).

In the total histones of the cotton plant (Fig. 3A), as in the calf-thymus histones (Fig. 3B) there are six main fractions, but in the nature of their positions they differ considerably. The only exception is the arginine-rich H4 histone. The histones of both types migrate in the given electrophoretic system as single zones (apart from the H1 fraction, which separates into two components).

The results that we have obtained on the properties of the cotton-plant histones agree to a certain extent with modern ideas on the conservatism of individual histone fractions. However, on comparing the electrophoretic mobilities of the histones under consideration it

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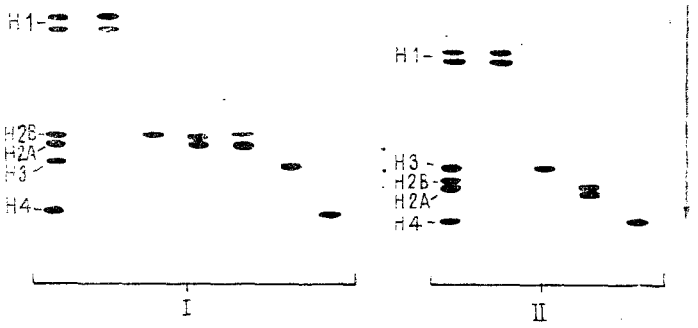

Fig. 1

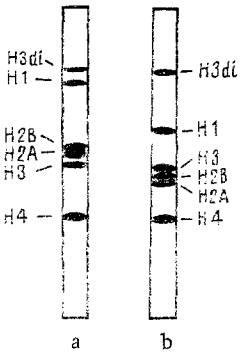

Fig. 2

Fig. 1. Electrophoresis of cotton-plant histones (I) and calf thymus histones (II) in a block of gradient PAAG with Na-DDS after fractionation on a column of Acrilex P-60.

Fig. 2. Electrophoretic separation of the total cotton-plant histones (a) and the total calf-thymus histones (b) in an acid-urea gel [2].

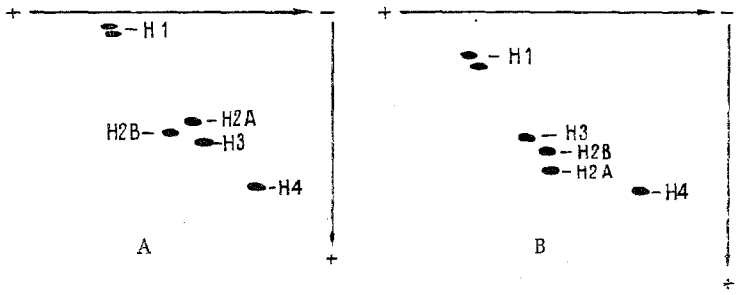

Fig. 3

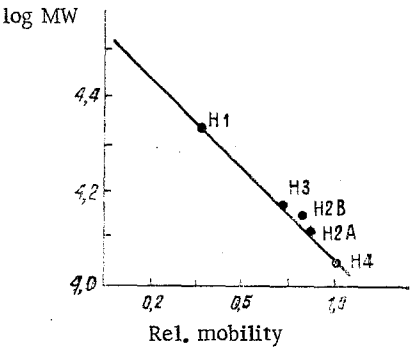

Fig. 4

Fig. 3. Two-dimensional electrophoresis of cotton-plant and calf-thymus histones (A) and (B) respectively.

Fig. 4. Calibration curve of the dependence of the mobility of standard proteins in gradient PAAG containing Na-DDS on their molecular weights.

may be assumed that the histones of the cotton plant possess somewhat higher molecular weights than the calf-thymus histones.

We used a comparative evaluation of the electrophoretic mobilities of the histones in a block of gradient PAAG in the presence of Na-DDS to determine the molecular weights of the cotton-plant histones. Because of features of the structure of the histones and their large charge, the calibration graph (Fig. 4) was constructed on the basis of standard proteins belonging to the same class, i.e., the calf-thymus histones. Below we give the molecular weights of the cotton-plant histones determined from their relative mobilities in PAAG and of the calf-thymus histones:

Fraction	Mol. wt. of the histones	
	of calf thymus, calculated from their primary structures [5-9]	of the cotton plant (our results)
H1	21 000	23 500
H2A	14 231	14 600
H2B	13 770	14 960
H3	15 220	14 300
H4	11 300	11 300

Fractions H1, H2B, and H2A of the cotton-plant histones have larger molecular weights than the corresponding fraction of the calf-thymus histone.

The results obtained confirm the hypothesis put forward previously that plant histones differ from animal histones by greater dimensions of the molecules and are in harmony with the results of a comparative study of the histones of rye and the calf thymus [4].

EXPERIMENTAL

The total histones and individual fractions of the histones of the cotton plant of variety 108-F and of the calf thymus were used. The conditions for isolating and fractionating the histones have been described previously [1].

Electrophoretic Investigations of the Histones. Electrophoresis in the presence of Na-DDS was carried out by a modification of the method of Thomas and Kornberg [3] in a vertical block with dimensions of 150 × 130 × 1.5 mm at a voltage of 60 V for 16-20 h. The total histones were deposited in an amount of 25-30 μg and the individual fractions in amounts of 5-7 μg per well. The course of electrophoresis was monitored from the movement of the dye bromophenol blue. The PAAG block was fixed in 20% trichloroacetic acid for 30 min and was stained with a 1% solution of Coomassie brilliant blue R=250 in 50% methanol containing 5% of CH_3COOH for an hour. The background was decolorized by the repeated washing of the gel blocks with 7% CH_3COOH or with methanol—water—CH_3COOH (5:5:1).

Two-dimensional electrophoresis was performed in the vertical direction by the method of Panyim and Chalkley [2] in tubes 7.5 cm long at a current strength of 1.5 mA per tube, and in the horizontal direction by the method of Thomas and Kornberg [3] in a PAAG block with dimensions of 150 × 100 × 2 mm at a voltage of 120 V for 16-20 h. The block was stained for 1.5-2 h.

The calibration graph for determining the molecular weights of the histones of the cotton plant was plotted from the figures for calf-thumus histones with known molecular weights [5-9].

CONCLUSION

1. The fractional composition of cotton-plant histones has been studied by electrophoresis in a block of gradient polyacrylamide gel in the presence of sodium dodecylsulfate. It has been established that the cotton-plant histones contain five main fractions which differ in their rates and sequence of migration in polyacrylamide gel from the corresponding fractions of calf thymus histone.

2. The molecular weights of the cotton-plant histones have been determined.

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