CONCLUSIONS

The redistribution of electron density and also the change in the energies of the lowest singlet and triplet excited states in quinone and quinone methides depends to a considerable degree on the chromophores present in them. The substantial differences in these properties permit the assumption of dissimilar participations of the chromophores under consideration in the transformation processes of lignin. A change in the donor-acceptor properties of the fragments in excited states leads to a change in the pathways of electrophilic and nucleophilic reactions as compared with the ground state.

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COMPETITIVE BINDINGS OF HISTONES WITH DNA IN FORMATION OF PLANT NUCLEOHISTONES

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The nature of the relative affinity of histones for DNA has been studied with the aid of the electrophoretic analysis of the DNP complexes obtained by the addition of the total histone to a nuclear histone with a DNA:histone ratio of 1:1. It has been established that, on the addition of histone, complexes are formed the composition of which changes according to the degree of competition of the histone fractions. Some differences have been detected in the nature of the formation of nuclear histones of plant and animal origin due to features of the primary structure of the histones. The results obtained confirm a hypothesis put forward previously about the different strengths of the bonds of histones with DNA and the role of dissociation in the functioning of the genetic apparatus of the cells of higher organisms.

Advances of recent years in the study of the structural-functional state of chromatin have considerably promoted our understanding of the mechanisms of the functioning of the genetic apparatus of eukaryotes. General features of the nucleosomal organization of the chromosomal fibrils have become known [1, 2]. Information has been obtained on the nature of the packing of the nucleosomes into structures of higher order [3-5]. However, so far the role of certain histones and nonhistone proteins in the packing of DNA has been unclear. There is little information on the structural organization of the chromatin of higher plants [6].

The study of the main components of chromatin and the elucidation of the nature and specificity of their interaction form one of the key points in the solution to these questions.

In connection with a comparative study of the histones of the calf thymus and the cotton plant — objects remote from one another in the evolutionary series — and in order to deter-

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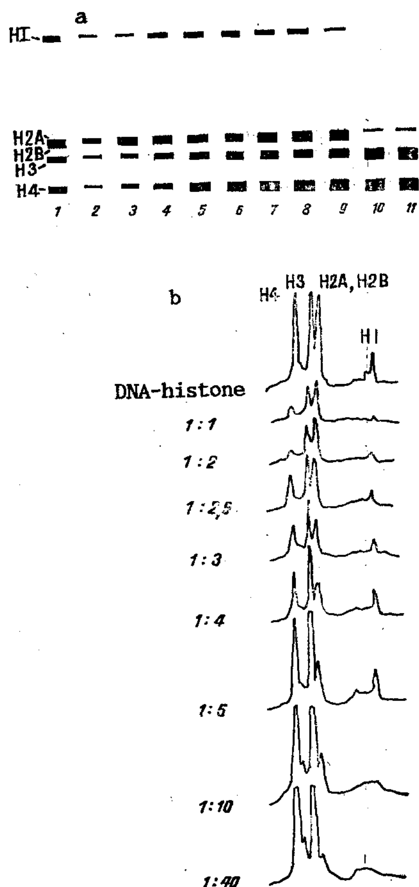

Fig. 1. Electrophoretogram (a) and densitogram (b) of histones present in a complex with DNA. Ratio of DNA to histone in the mixtures, by weight: 1) total cotton-plant histone (control); 2) 1:1 (initial nucleohistone); 3) 1:1.5; 4) 1:2.0; 5) 1:2.5; 6) 1:3.0; 7) 1:4.0; 8) 1:5.0; 9) 1:10; 10) 1:20; 11) 1:40.

mine a functional anomaly, it appeared of interest to study the competitive binding of histones with DNA that was observed in the self-assembly of chromatin [7, 8] using cotton-plant histones and DNA as an example.

For the investigation we isolated highly polymeric nuclear DNA from 2-day cotton seedlings by a method suggested previously [9], and histones by acid extraction from the nuclei followed by stages of dialysis and lyophilization [10]. However, under the conditions of performing the experiment (0.15 M NaCl + 0.7 mM Na phosphate, pH 7.0) a considerable part of the cotton-plant histones, in contrast to the histones from the calf thymus, formed insoluble aggregates consisting of the histones of the nucleosomal nuclei. Electrophoretic analysis of the protein composition of the deposit and of the supernatant liquid (SL) after centrifugation of the histone solution showed that the deposit contained the H2A, H2B, H3, and H4 fractions and lacked H1, while the SL contained the H1, H2A, H2B fractions, together with H3 and H4 in trace amounts. Such behavior of histones indicates irreversible structural changes taking place during their isolation by acid extraction. The main causes of the structural changes of histones may be dephosphorylation of the lysine and histidine residues, deamination of asparagine and glutamine residues, and a number of other postsynthetic modifications affecting histone-histone interaction [11]. It was also established that the freeze-drying of the proteins led to irreversible structural changes [12]. In order to increase the degree of native nature and the purity of the cotton-plant histones, we modified the method of isolation by excluding the stage of freezing and lyophilization. Under conditions close to physiological, the preparation of total histone isolated in this way contained all five fractions and was used in the subsequent experiment.

The nature of the relative affinity of the histones for DNA was studied with the aid of electrophoretic analysis of the DNP complexes obtained by the addition of an excess of total histone to a nucleohistone, with a histone:DNA ratio of 1:1, in a medium with the physiological ionic strength. Figure 1 shows an electrophoretogram and densitogram of the histones present in the deposits after centrifugation of mixtures with DNA:histone ratios of from 1:1 to 1:40. As can be seen, the addition of the total histone to the nucleohistone led to the formation of DNP complexes the compositions of which changed according to the degree of competition of the histone fractions for the DNA. Under the given conditions,

complexes were formed which contained the histones of the nucleosomal core — H2A, H2B, H3, and H4 — and then complexes containing only the arginine-rich fractions H3 and H4. The observed sequence of displacement of histones from complexes, first of H1 and then of H2A and H2B permits the cotton-plant histones, like calf-thymus histones [7], to be arranged in order of the strength of their bond with DNA in the following sequence: H1 < H2A + H2B < H3 + H4. However, some differences were observed in the nature of the formation of the plant nucleohistones. As the total histone was added, the formation of complexes with changed amounts of individual fractions took place. At a DNA:histone ratio of from 1:1 to 1:2, the complex was enriched with the H2A and H2B fractions, while at higher ratios the amount of the arginine-rich fractions H3 and H4 increased. The sequence of displacement of the cotton-plant H1, H2A, and H2B was observed only with a tenfold excess of total histone with respect to the DNA, while the corresponding calf thymus histones were displaced from the complex at a fivefold excess of histone [7, 8]. The lysine-rich histone H1 of the cotton plant was displaced from the complex completely at a 20-fold excess of the total histone, and the moderately lysine-rich H2A and H2B were present in the complex in small amount (2%) at a 20- and even at a 40-fold excess. The facts indicate that the histones of the cotton plant have a greater affinity for DNA than the histones of the calf thymus.

For the case of the comparative study of the competitive binding of histones with DNA it has been shown that the formation of nucleohistones of plant and animal origin is based on common principles. However, some differences are observed in the nature of the formation of the plant complexes which are due to features of the primary structure of the histones. The results obtained confirm the hypothesis put forward previously on the different strengths of the binding of histones with DNA and the role of the dissociation of the histones in the functioning of the genetic apparatus of the cells of higher organisms.

EXPERIMENTAL

The total histone from a cotton plant of the 108-F variety was isolated from the cell nuclei of 2-day seedlings by acid extraction [10] and was precipitated with a tenfold volume of cooled acetone and left at −20°C for 24 h. The solution was centrifuged at 10,000 rpm, and the deposit was washed twice with cooled acetone and was dried in vacuum. The histones were dissolved in 0.7 mM sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl, and the solution was centrifuged at 40,000 rpm for 30 min. The amounts of histones in the deposit and the supernatant liquid were determined with the aid of electrophoresis in PAAG with Na DDS [13]. The supernatant liquid was used for preparing histone-DNA complexes. Nuclear DNA was isolated from cotton-plant seedlings [9], depolymerized in an ultrasonic disintegrator, and mixed with the total histone in a ratio of 1:1. Additional amounts of total histone were added to the nucleohistone obtained in this way, and the mixtures with different DNA: histone ratios, from 1:1 to 1:40, were incubated at 4°C for 24 h and were then centrifuged at 40,000 rpm for 60 min. The precipitates obtained after centrifugation were used for the analysis, by electrophoresis in PAAG [13], of the histones bound to the DNA. The amounts of protein in the deposits and the SL were determined by Lowry's method [14], and the DNA by Spirin's method [15]. The densitometry of the electrophoretograms after the gels had been stained with Coomassie Blue R-250 was carried out on an Ultraoscan 2202 spectrometer (Sweden).

CONCLUSIONS

1. The nature of the relative affinity of histones for cotton-plant DNA has been studied.

2. Differences have been found in the nature of the formation of plant nucleohistones.

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