ISOLATION AND PHYSICOCHEMICAL CHARACTERIZATION OF HIGHLY POLYMERIC COTTON-PLANT NUCLEAR DNA

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UDC 547-963.3;633.51.581.19

A highly polymeric fraction of nuclear DNA has been isolated from two-day shoots of the cotton plant. The DNA has been characterized by the methods of circular dichroism (CD), thermal denaturation, and ultracentrifugation. It has been established that the DNA preparation isolated has a molecular mass of $18 \cdot 10^6$ daltons, melts at 85.5°C in a fairly narrow interval, and reveals no heterogeneity of its intramolecular nucleotide composition. It has been shown by the CD method that in a solution of the cotton-plant DNA with relatively low ionic strength there is a tendency to the transformation of the B form into the C form.

Considerable attention is being devoted to the study of the nucleic acids of higher plants [1-3]. An investigation of the structure of DNA is important for understanding the principles of the organization of the genome in plants and also the mechanisms of reproduction and of the realization of genetic information.

The isolation of highly purified genetic material degraded to the minimum extent is an important step. However, no universal method of isolating DNA from higher plant exists, since it is necessary to face the methodological difficulties connected with the breakdown of the cells and with the large amount of impurities interfering with purification and leading to a low yield of DNA and other substances.

One complex and little-studied object is the cotton plant. Investigations [4, 5] have been devoted to the study of cotton-plant DNA and the determination of its nucleotide composition.

The aim of the present investigation was to isolate highly polymeric nuclear DNA from the cotton plant and to characterize it by using the methods of circular dichroism (CD), heat denaturation, and ultracentrifugation.

To isolate the nuclear DNA we used Marmur's method [6] with certain modifications for plants [7]. The initial stage, including the isolation of highly purified nuclei, was carried out by a modified Alfrey method [8]. The purity of the nuclei was checked with the aid of a ML-3 luminescence microscope.

The results of the investigation showed that the method used by us enabled a sufficient amount of satisfactorily purified and minimally degraded DNA preparation to be obtained. The amount of protein and RNA did not exceed 1% and the 260/280 nm and 260/230 nm ratios were $\geqslant 2$. The hyperchromic effect of the DNA measured in salt solution was 39-40%. The degree of nativeness (E_p) of the cotton-plant DNA in solution with different concentrations of salts remained constant and equal to 6500. It follows from this that an increase in the ionic strength had no denaturing action on the DNA at room temperature and a neutral pH.

The molecular weight of the nuclear DNA was determined by the ultracentrifugation method [9]. It was established that the isolated DNA preparation had a high molecular mass — $18 \cdot 10^6$ daltons.

A study of the thermal melting curves of the cotton-plant DNA (Fig. 1a) in 0.1 M phosphate buffer showed that the DNA melted as a single component in a fairly narrow interval and did not show a heterogeneity of the intramolecular nucleotide composition. The melting point of the DNA was 85.5°C and the width of the peak interval (ΔT) 6°C.

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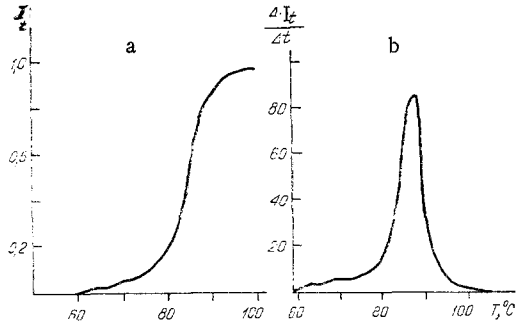

Fig. 1. Melting curves of cotton-plant nuclear DNA in 0.1 M phosphate buffer: a) integral form of the curve where $I_t = \frac{D_{260}^t - D_{260}^{min}}{D_{260}^{max} - D_{260}^{min}}$ (D_{260}^{min}, D_{260}^{max} are the minimum and maximum values of the optical density of the DNA solution at 260 nm and D_{260}^t is the optical density of the solution at the given temperature); b) differential form of the curve, rate of heating 1°C/min.

In order to study the possible heterogeneity of the DNA, the integral form of the curve was converted into the differential form (Fig. 1b). The amount of CG pairs of nucleotides calculated from the melting points by means of Marmur and Doty's formula [10] was 39 mole-%, which permitted the cotton-plant DNA to be assigned to the AT type.

The most unambiguous information on the structure of a DNA can be obtained with the aid of the CD method. The study of the CD spectra of polynucleotides and nucleic acids [11-14] has shown that in the region of the spectrum above 220 nm DNA has two CD bands approximately equal in magnitude due to the $\pi \rightarrow \pi^*$ transition in the nitrogen bases: positive with a maximum at 272 nm and negative with a maximum 245 nm. With an increase in the ionic strength of the solution there was a shift in the maximum of the positive CD band in the direction of longer wavelengths. The amplitude of the negative band also increased and its maximum shifted in the long-wave direction. Such behavior of the CD of the DNA is similar to that of the CD of DNP complexes and, consequently, the histones in the DNP exert the same action on the structure of the DNA as a high ionic strength of the solution. A change in the ionic strength leads to conformational rearrangements of DNA with no disturbance of the integrity of the double helix [15].

The CD spectrum of the cotton-plant DNA (Fig. 2a) taken in 0.1 M phosphate buffer at pH 7.0 was similar to the CD spectrum of calf thymus DNA in a solution with a low ionic strength, when it is present in the B form, with a positive maximum in the 272-280-nm region and a negative maximum in the 245-nm region (Fig. 2b). However, in the CD spectrum of the cotton-plant DNA the intensity of the negative peak was higher than that of the positive peak and a shift in the long-wave direction of the positive maximum was observed. These rearrangements apparently amount to some rotation of the bases and a change in the number of bases per turn of the helix.

The results obtained permit the assumption that for the cotton-plant DNA in a solution with a relatively low ionic strength there was a tendency for the conversion of the B form into the C form (Fig. 2c). This behavior of the cotton-plant DNA indicates some properties distinguishing the plant DNA from the calf thymus DNA and requires a more detailed study.

EXPERIMENTAL

Isolation of the Cotton-Plant Nuclear DNA. The DNA was isolated from two-day shoots of cotton plants of the variety 108-F by Marmur's method [6] with some modifications [7]. The initial stage — the isolation of the nuclei — was performed by Alfrey's method [8] with slight modifications. The fresh plant material was ground at 4°C in a mortar with buffer A (0.25 M sucrose, 0.004 M $MgCl_2$, 0.004 M $CaCl_2$, 0.05 M Tris-HCl, 0.005 M $Na_2S_2O_5$) (pH 7.0). The resulting suspension was homogenized in the same buffer at 2000 rpm for 50 sec and was filtered through two layers of glass and eight layers of Kapron [nylon-6].

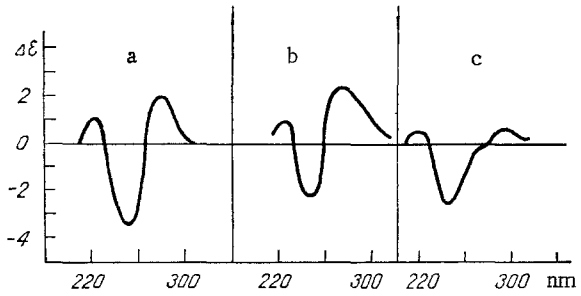

Fig. 2. CD spectra: a) cotton-plant DNA in 0.1 M phosphate buffer at pH 7.0; b) calf thymus DNA in the B form; c) calf thymus DNA in the C form.

To separate and purify the nuclei, the homogenate was centrifuged at 2200 rpm for 20 min. The nuclei were subjected to additional purification by washing with buffer A containing 1% Triton X-100 and were centrifuged in a 1.7 M sucrose gradient at 3000 rpm for 20 min.

After repeated washing with citrate buffer (pH 7.0), the nuclei were suspended in buffer B (0.05 M Tris-HCl, 0.1 N EDTA, 0.15 M NaCl, pH 8.0) and were lysed with a 2% solution of Sarkosyl [lauroylsarcosine] at 37°C for 30 min. Then NaCl was added to give a final concentration of 1 M and the lysate was deproteinized twice by shaking with an equal volume of a mixture of chloroform and isoamyl alcohol in a ratio of 24:1. Traces of RNA were eliminated by RNase treatment (50 μg/ml, 37°C, 1 h). The RNase had previously been heated to 80°C. In addition, the material was treated with pronase that had previously been heated to 50°C. After several deproteinations, the DNA was precipitated with two volumes of cooled 96% ethanol and was stored in 75% ethanol at −10°C.

Melting of the DNA. The thermal denaturation of the nuclear DNA was performed in a SF-4 instrument with a thermostated device in the cell compartment in 0.1 M phosphate buffer, pH 7.0. The concentration of DNA was 25 μg/ml.

The molecular weight of the DNA was determined by the ultracentrifugation method on a Spinco E instrument as described by Golfrey [9] in 2 mM phosphate buffer containing 0.2 M NaCl, 2 mM EDTA, pH 7.0.

The CD spectra of the cotton-plant DNA were recorded on a Cary-6003 spectropolarimeter with an attachment for measurement in the 320-210-nm region in a thermostated cell with a path length of 1 mm. The absorption of the cuvette with the solution did not exceed 1.5 optical density units.

CONCLUSION

The highly polymeric nuclear DNA of the cotton plant has been isolated and characterized, and it has been shown that in a solution with a relatively low ionic strength there is a tendency for the B form of the DNA to undergo transformation into the C form.

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AN INVESTIGATION OF THE COMPOSITION OF AN ETHEREAL EXTRACT OF THE FRUIT STONES OF *Anisophyllea laurina*

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UDC 543.8+547.426.23+665.3

It has been shown by the methods of IR and ^1H and ^{13}C NMR spectroscopy and also by spectrometry that ether extracts a fat from the kernels of the fruit stones of *Anisophyllea laurina* (family *Rhizophoraceae*, Guinea). The amount of fat is about 16% of the weight of the kernels. The acid composition of the fat has been studied by gas chromatography and mass spectrometry. It contains the following acids (%): myristic (traces), palmitoleic (26.1), palmitic (54.4), linoleic (5.5), oleic (10.1), vaccenic (3.1), and stearic (0.8).

The drupaceous tree *Anisophyllea laurina* (family *Rhizophoraceae*) the fruit of which is used as food, grows in west Africa, including Guinea. The stones of the fruit have never yet been used. In order to determine the possibility of their utilization, we have investigated the composition of an ethereal extract of the kernels of the stones. After evaporation of the ether, a white solid was obtained (about 16% of the weight of the kernels) which after some days changed into a brownish pasty mass. Recrystallization of this mass from a mixture of methanol and acetone gave a white substance which did not change on prolonged storage.

It was shown by the methods of elementary analysis, IR spectroscopy, and ^1H and ^{13}C NMR spectroscopy that the purified substance consisted of tripalmitin while the initial unpurified product contained free acids, in addition to glycerides of carboxylic acids. From the ratio of the intensities in the IR spectrum of the absorption bands of the carbonyl groups of the glycerides ($\nu_{C=O}$ = 1740 cm^{-1}) and of carboxylic acids ($\nu_{C=O}$ = 1720 cm^{-1}) it followed that the amount of the latter in the initial material was 25-30%. The presence of free acids in the unpurified material is most probably due to the splitting of triglycerides taking place during storage.

A mass-spectrometric analysis of the initial material showed that it contained glycerides including residues not only of palmitic acid but also of hexadecenoic, octadecadienoic, octadecenoic, and octadecanoic acids. They were identified by the ions of the corresponding acyloxy residues (m/z = 253, 255, 279, 281, and 283).

The acid composition of the glycerides of the stones of *Anisophyllea laurina* was studied in more detail by converting the unpurified material into methyl esters and analyzing the latter with the aid of chromato-mass spectrometry and gas chromatography. The chromato-mass spectrometric analysis of the methyl esters confirmed the acid composition of the initial material given above. With the aid of this method it was also shown that the material contained trace amounts of tetradecanoic acid.

The mass-spectrometric analysis did not give unambiguous information on the structure of the skeletons of the acids or on the positions of the multiple bonds in the unsaturated acids. The acids present in the initial material were therefore identified in the form of their methyl esters by comparing their Kovats indices (Table 1) with the corresponding indices of standard esters and those given in the literature [1]. The close values of the indices

M. V. Lomonosov Moscow State University. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 645-647, September-October, 1984. Original article submitted February 1, 1984.