MELTING PROFILES OF LYMPHOCYTE CHROMATIN IN DOWN'S SYNDROME DETECTED BY AN OPTICAL DENSITY TECHNIQUE

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Previously [3, 4] the authors showed by luminescence microscopy with acridine orange (AO) on a short-term culture of human cells that the melting profiles of chromatin from intact healthy human lymphocytes consist of curves with maxima (F_{530}) in the region of particular temperatures: 45, 55, 65 (±3), 78, 85, 88, and 92 (±2)°C. Melting profiles of lymphocytes from patients with Down's syndrome are curves with maxima also in the region of particular temperatures: 65, 85, 88, and 92 (±2)°C.

The decrease in the number of maxima, the merging of two maxima (78 and 85°C) into one confluent maximum in the region of 85°C, the absence of a maximum at 45°C, and also a decrease in the relative value (F_{530}) of all peaks in all cases suggested an increased degree of condensation of nuclear chromatin in cells of patients with Down's syndrome.

Since the main "diagnostic" test used to investigate the structure of the chromatin complex of human cells with the aid of AO in order to analyze the data was the melting profiles of deoxyribonucleoproteins (DNP) of the cells (which are curves with maxima in the region of particular temperatures), the object of the present investigation was an experimental attempt to study the nature of the maxima observed. Physicochemical modifications both of the nucleoprotein isolated and of the chromatin complex of intact cells under the influence of temperature can be caused by two main factors: 1) dissociation of protein or labilization of its bond with DNA and 2) in the case of chromatin, differences in the thermostability of different regions of DNA [1, 7].

The object of the present investigation was to study the state of the secondary structure of DNA of the cell chromatin and the possibility of its effect on structural modifications of cell DNP under the influence of temperature under normal and pathological conditions.

EXPERIMENTAL METHOD

A comparative analysis was made of temperature-dependent changes in absorption of chromatin DNA (at 260 and 320-340 nm) from lymphocytes of healthy human subjects and patients with trisomy-21, during heating the cells in media with different NaCl concentrations (0.015, 0.0015, and 0.00015 M) at pH 7.0. Media of low ionic strength for melting the cells were used in order to abolish the specific supramolecular packing of cell DNP which otherwise exists [5, 6]. The absorption curve of cell DNA in the ultraviolet region during melting in medium with physiological ionic strength (0.15 MNaCl), pH 7.0, served as the control; measurements were made at 5°C intervals within the temperature range 20-100°C.

Altogether 30 experiments were carried out on lymphocytes from 20 healthy subjects (10 women and 10 men aged 20-36 years) and 10 patients with Down's syndrome (5 women and 5 men aged 16-26 years). Methods of taking blood and preparing specimens for cytophotometric analysis were described previously [3, 4]. Terminal denaturation of chromatin in the cells was carried out by a method modified by the authors [2, 4]. To prevent renaturation of the DNA, after heating in the appropriate solution for 20 min at a certain temperature, the slides with the adherent cells were transferred into two parallel fixing solutions: 1) a mixture

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(acetone:ethanol, 1:1) previously cooled to between −5 and −7°C; 2) 4% solution of formaldehyde. No difference was found in the results as a result of fixation of the cells by different methods.

Measurements were made on a microscope-photometer (from Opton, model 0.5). The diameter of the probe was commensurate with the dimensions of the cell. An integral value of optical density (E) of not less than 10 cells was obtained; at each temperature the nonspecific scattering of light at 320 nm (E_{320}) was about 5% of E_{260} and, consequently, no significant corrections were introduced into the principal results. Chromatin DNA melting profiles from cells of the healthy subjects and patients were compared at each temperature point studied (12-15 points) with respect to two indices; the mean intensity of absorption and the parallelness of the change in its intensity. The significance of divergence from parallelness was evaluated as the significance of the difference of variation of partial differences from random scatter [2].

EXPERIMENTAL RESULTS

Changes in optical density of healthy human lymphocyte nuclei during heating in solution with physiological ionic strength (0.15 M NaCl, pH 7.0) are shown in Fig. 1. A significant hyperchromic effect by 35-40% ($P < 0.01$) was observed at 260 nm, starting from 78°C, reaching a plateau by 82-85°C and remaining at that level until 96°C.

Absorption of total protein at 230 nm on the whole repeated the configuration of the curve obtained at 260 nm. In 5 of the 20 experiments the hyperchromic effect was considerably reduced (7-15%). It should be noted that in these subjects different deviations in the distribution of the maxima on the cell DNP melting curve obtained with the aid of AO were observed.

When the cell DNA was melted in media with low ionic strength (0.00015 M NaCl) the optical density of the nucleus remained unchanged up to 96°C. These results are shown graphically as a straight line (Fig. 1D). At higher ionic strengths (0.015 and 0.0015 M NaCl) a tendency for the optical density to rise by 5-7% ($P < 0.05$) was observed, starting from 78-82°C (Fig. 1B, C). Identical results were obtained in 15 of the 20 experiments.

During melting of the aneuploid cells of patients with Down's syndrome in 0.15 M NaCl the optical density of the nuclei studied remained unchanged up to 98°C regardless of the method used to fix the cells after heating (Fig. 2). Identical results also were obtained when trisomic cells were melted in media with low ionic strength. For instance, the integral values of optical density of the nuclei showed that melting cell chromatin DNA from healthy subjects begins at 78-80°C, rises gradually to 35-40%, and reaches a plateau at 96°C. A hyperchromic effect on aneuploid cells under similar experimental conditions was observed only after 98°C.

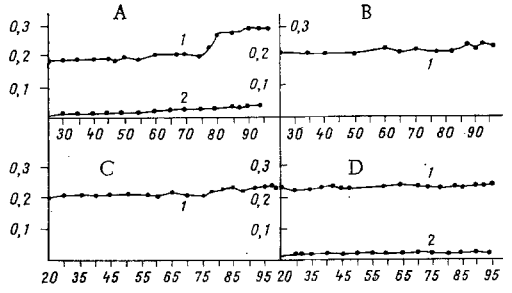

Fig. 1

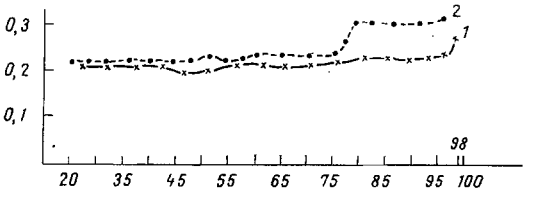

Fig. 2

Fig. 1. Optical density (E_{260}) of DNA from healthy human peripheral blood lymphocytes during melting of the cells in media with different ionic strengths. Melting medium for cells: A) 0.15 M NaCl, B) 0.015 M NaCl, C) 0.0015 M NaCl, D) 0.00015 M NaCl. 1) A_{260}, 2) A_{230}. Abscissa, temperature (in °C); ordinate, E_{260} (in relative units).

Fig. 2. Optical density (E_{260}) of nuclei of peripheral blood lymphocytes from patients with Down's syndrome when cells were melted in medium of physiological ionic strength (0.15 M NaCl). 1) Down's syndrome, 2) healthy subjects (control). Remainder of legend as in Fig. 1.

To what can this "delay" of the hyperchromic effect be attributed? The optical density of the nucleus is known to be determined by the state of the secondary structure of DNA which, in turn, in chromatin depends on the character of its bond with the surrounding proteins. The absence of an increase in the absorption of DNA in the composition of the modified chromatin of trisomic cells up to 80°C was evidently due to the fact that this temperature is too low to rupture the bonds between the DNA chains and to convert it into the "coiled" state. These data confirm our idea of the high degree of condensation of the aneuploid genome.

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GENETIC FEATURES OF F-LIKE PLASMID pAP10-2 CONTROLLING SYNTHESIS OF THERMOSTABLE ENTEROTOXIN BY *E. coli* CELLS

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During an investigation of the plasmid complex discovered by the writers previously [1] in cells of conditionally pathogenic strain *E. coli* AP42-1 (serogroup 0101), isolated from a diseased calf, plasmid pAP10-2 with a molecular weight of 65 megadaltons, controlling synthesis of a thermostable enterotoxin (Ent-plasmid) was identified.

The object of this investigation was to study the genetic features of this Ent-plasmid after transmission into cells of plasmid-free strains of *E. coli* K-12.

EXPERIMENTAL METHOD

The ability of bacteria to produce thermostable enterotoxin was determined by intraperitoneal injection of 0.1 ml of cell-free culture fluid into newborn suckling mice [7]. The mice were kept for 4 h at 30°C and then killed, and their small intestine was weighed. The results of the experiments were expressed as a gravimetric index (ratio of weight of intestine to weight of remainder of animal). An isogeneic plasmid-free strain of *E. coli* K-12 was used as the control.

Other genetic markers were studied and the test bacteria conjugated by standard methods [1]. The sensitivity of the bacteria to donor-specific phage MS2 was determined by the agar layers method [8] or by tests to determine the increase in titers of this phage [3]. To eliminate individual plasmids the bacteria were grown in the presence of ethidium bromide [6].

Genetic marking [11] of the test plasmid was carried out with transposon Tn9, carrying determinants of resistance of the bacteria to chloramphenicol, and a component of plasmid RP1 with a temperature-sensitive replication system (a strain of bacteria containing this plasmid

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