STRUCTURE OF INTERPHASE CHROMATIN OF HUMAN DIPLOID AND TRISOMIC CELLS STUDIED BY THERMAL DENATURATION IN MEDIA WITH LOW IONIC STRENGTH

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The writers previously showed [3, 4] by the use of luminescence microscopy with acridine labeling on short-term cultures of human cells, in their suggested modification of the thermal denaturation method, that melting profiles of interphase chromatin of intact lymphocytes from healthy subjects consist of curves with six maxima (F_{530}) at definite temperatures — 45, 65, 78, 85, 88, and 92°C (±2°C), whereas those of patients with Down's syndrome are curves with three maxima at temperatures of 65, 85, and 92°C (±1°C).

The object of this investigation was to study the nature of the peaks arising under the influence of temperature on the curve of structural changes in chromatin of human cells.

EXPERIMENTAL METHOD

A technique abolishing the regular supramolecular packing of the DNP-complex of the cells [9] by incubating them in nonphysiological media, i.e., in media with below physiological ionic strength, was used. Comparative analysis of temperature-induced changes in acridine orange binding with DNP of human lymphocytes during heating the cells in media with different NaCl concentrations (0.015, 0.0015, 0.00015 M) of low ionic strength, pH 7.0, was carried out. The curve of structural transitions of the cell chromatin during exposure to heat in a medium with physiological ionic strength (0.15 M NaCl), pH 7.0, served as the control. According to the now generally accepted view, the dye acridine orange (AO), when bound with intact double-stranded DNA, fluoresces with a maximum of emission at λ = 530 nm, whereas the dye bound with denatured DNA has an eimssion maximum at λ = 640 nm. The ratio of the intensity of fluorescence of the AO-DNA complexes at λ = 640 nm and λ = 530 nm (α) is the degree of orderliness of the DNA in the DNP-complex, or in this case in the cell [5, 8], and this was adopted as criterion of the structural state of DNA of the cell chromatin.

The methods of taking blood, making the preparations for microcytofluorometric analysis, and the parameters of the arrangements in which the measurements were carried out were described previously[3, 4]. Thermal denaturation of chromatin in the cells was carried out by Ringertz' method [8] in the writers' own modification [3]. Before turning to the study of melting profiles of chromatin in media with reduced NaCl concentrations, the quantity of dye bound with cell chromatin in media of different ionic strengths at 20°C, with subsequent fixation (acetone—ethanol, 1:1) and staining in a solution of AO in 015 M NaCl, was determined (by measuring the intensity of fluorescence). No difference was found in the ability of the chromatin to bind AO, i.e., in all four investigations there was no change in F_{530}, which was equal to 1 (relative units); F_{640} likewise was unchanged and equal to 0.15 relative unit; correspondingly there was no change in α.

The melting profiles of chromatin of the test cells cultured under different conditions were compared at all points of the melting curve with respect to the mean intensity of fluorescence and the parallelness of the change in its intensity. The significance of differences was estimated by a special version of two-factor dispersion analysis. The significance of the difference in the mean level of the processes was determined as the significance of the difference from zero of the mean difference of the partial means, and the significance of nonparallelness of divergence was determined as the significance of the difference in variation of partial differences from the random scatter [1].

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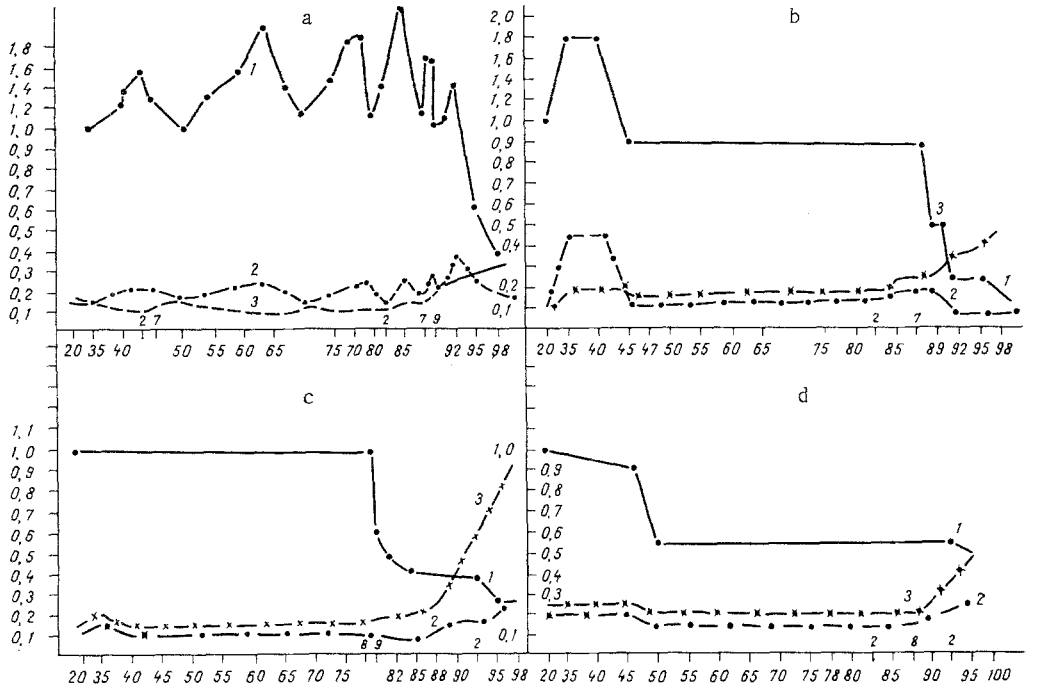

Fig. 1. Melting profiles of chromatin of healthy human lymphocytes obtained by luminescence microscopy with acridine orange (F_{530}; F_{640}; $F_{640}/F_{530} = \alpha$) in media with different ionic strengths, pH 7.0. Abscissa, temperature (in °C); ordinate, ratio of intensity of fluorescence of AO at the given temperature to that at 20°C. Here and in Fig. 2: a) medium for melting cell chromatin 0.15 M NaCl (control); b) 0.015 M NaCl, c) 0.0015 M NaCl, d) 0.00015 M NaCl. 1) F_{530}; 2) F_{640}; 3) α.

EXPERIMENTAL RESULTS

Healthy Subjects

1) 0.15 M NaCl, pH 7.0 (Fig. 1a) — control. A significant increase in the intensity of fluorescence of the dye by 2-2.5 times was observed in the region of temperatures of 45, 65, 78, 85, 88, and 92 C (±2°C). Starting with 92°C, a fall in the value of F_{530} was observed. Fluctuations in the intensity of fluorescence in the F_{640} region were identical with changes in F_{530} up to 88°C, after which, repeating the profile of the F_{530} wave, the general level of F_{640} rose, to fall sharply after 92°C. The value of α increased from 88°C. Changes in F_{530} and F_{640} of the dye bound with DNA of the cell chromatin during melting in medium with 0.15 M NaCl were taken as the control.

2) 0.015 M NaCl, pH 7.0 (Fig. 1b). The melting profile of the cell chromatin as reflected in F_{530} was a curve with one maximum in the region of 35-40°C, a plateau up to 88°C, followed by a gradual fall of F_{530} at temperatures of 88, 89-90, and 92-94°C from 1 to 0.4 unit. F_{640} repeated changes in F_{530} in the melting profile up to 75°C, it rose slightly (by 0.1 unit) from 75 to 89°C, and then from 89 to 92°C it fell to 0.05 unit, and after 92°C it began to increase up to 0.25 unit. The value of α rose correspondingly from 72°C, with a sharp increase after 90°C.

3) 0.0015 M NaCl; pH 7.0 (Fig. 1c). In the region λ = 530 nm the structural transitions of the cell DNP were reflected in a curve with a plateau from 20 to 77°C, followed by a gradual fall in F_{530} at temperatures of 77, 79, 82, 88, and 92°C (to 0.25 relative unit). The configuration of F_{640} repeated the changes in F_{530} up to 77°C, from 77 to 82°C it fell to 0.1 relative unit, after which it began to increase up to 0.25 relative unit. The value of α increased gradually from 77°C, with a sharp rise from 88°C to reach the value of 1 after 98°C.

4) 0.00015 M NaCl, pH 7.0 (Fig. 1d). The values of F_{530} constituted a curve with a gradual fall in the intensity of fluorescence at temperatures of 45-50, 85, 92, and 98°C. Up to 87°C, changes in F_{640} repeated those in F_{530}, from 88°C it began to increase whereas F_{530} simultaneously fell. The value of α rose from 87°C to reach 1 at 98°C.

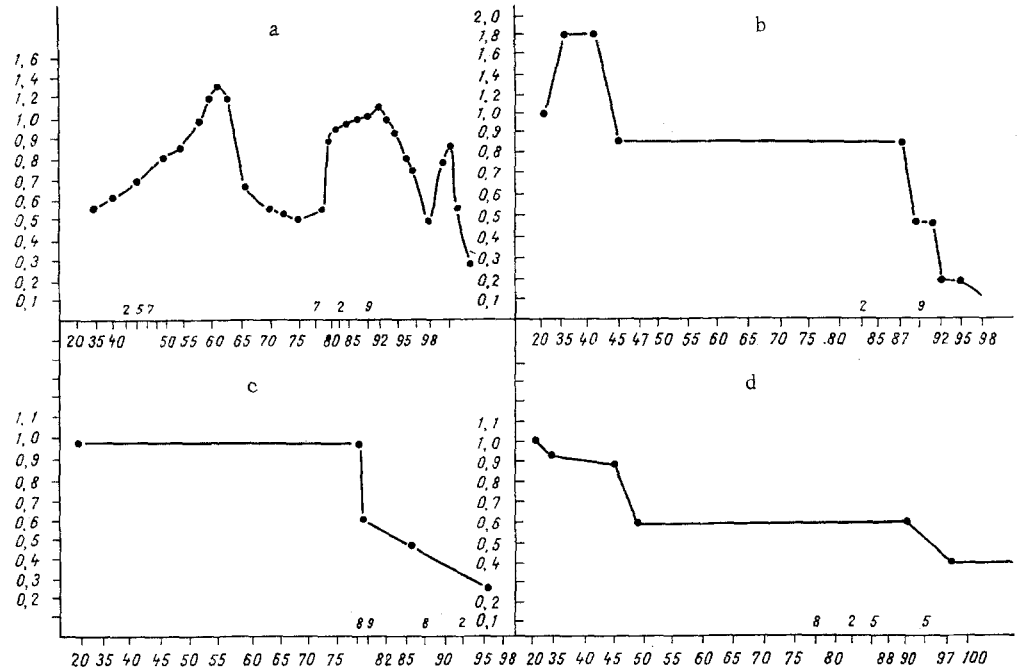

Fig. 2. Melting profiles of chromatin from lymphocytes of patients with Down's syndrome obtained by luminescence microscopy with acridine orange (F_{530}) in media with different ionic strength, pH 7.0.

Patients with Down's Syndrome.

When trisomic cells were heated in 0.15 M NaCl (pH 7.0) a significant increase was observed in the intensity of fluorescence of the dye by 1.5 times in the region of certain temperatures: 65, 85, and 92°C (±1°C), i.e., three maxima were obtained. Starting from 92-94°C the value of F_{530} was observed to fall. The fluctuations in the intensity of fluorescence of F_{640} were identical as regards melting profiles with those of F_{530}.

Changes in the intensity of fluorescence of AO bound with chromatin from trisomic cells on heating in 0.015, 0.0015, and 0.00015 M NaCl were identical with those obtained under corresponding conditions on intact healthy human cells (Fig. 2).

Melting cells in media with below-physiological ionic strength thus causes disappearance of maxima on the melting curve of the cell chromatin. In medium with ionic strength reduced by 1 order of magnitude below physiological (0.015 M NaCl) only one maximum remained, in the low-temperature region (35-42°C).

With a further decrease in ionic strength of the medium used to melt the cells (0.0015 and 0.00015 M NaCl) the profile of structural transitions in chromatin consisted of a curve without maxima, merely with steps of decrease of intensity of fluorescence at certain temperatures in both regions of the spectrum. Identical results were obtained on trisomic cells of patients with Down's syndrome when these cells were incubated in media with low ionic strength.

The results can be explained as follows. In medium of low ionic strength DNP-chromatin complexes are irreversibly unwound, specific DNA–protein bonds are replaced by strong salt-like bonds [2], and as a result the specific character of supramolecular packing of the chromatin, which evidently determines the origin of the peaks on the cell DNP melting curve, disappears. In other words the presence of maxima is a reflection of the superorganization of the supra-molecular packing of chromatin, evidently due mainly to the specific character of electrostatic (or hydrophobic [6]) DNA–protein and protein–protein interactions. It can be concluded from these findings that the action of high temperatures in conjunction with AO can reveal the specific character of supra-molecular packing of the DNP-complex of cells, which is preserved only in medium of physiological ionic strength; the packing of the superstructure of the cell chromatin of patients with Down's syndrome differs from that of the control.

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