INVESTIGATION OF RENATURATION OF THE DNP COMPLEX OF CELL NUCLEI BY A MICROFLUOROMETRIC METHOD

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A study of the kinetics of renaturation of DNP in the nuclei of human peripheral blood lymphocytes by means of a modified Rigler's method showed absence of renaturation of preparations previously heated to 65°C. Heating the preparations to higher temperatures (75, 85, and 100°C) led to the appearance of characteristic renaturation profiles with a maximum for incubation for 2 min (the intensity of fluorescence of the nuclei in cells transferred into cold salt-citrate solution was measured directly as a function of time).

Key words: nuclei of lymphocytes; reversible denaturation of deoxyribonucleoprotein; microfluorometric method of study.

A previous investigation [1] showed that DNA existing as a component of the deoxyribonucleoprotein (DNP) of individual cell nuclei, when denatured by heating (96-97°C, 20 min) in a medium containing 4% formaldehyde solution, can be rapidly renatured (after 1 min at 20°C, 50% renaturation is observed). The renaturation of DNA as a component of DNP thus differs significantly as a process from the renaturation of DNA in solution [4]. In the course of the investigation it was also discovered that a 4% formaldehyde solution does not prevent renaturation of DNA although it has a marked effect on the properties of the DNP complex.

It was decided to examine the kinetics of this very fast renaturation process. The study of renaturation of the DNP complex at the cell level could prove a useful clue to the understanding of the structure and properties of DNP and its components.

EXPERIMENTAL METHOD

Human peripheral blood lymphocytes were used to investigate the kinetics of renaturation of the DNP complex. A culture of white blood cells obtained by the method of Rigler and Killander [3] were fixed in ethanol–acetone (1:1) fixative for 1 h. The fixed cells were incubated in salt-citrate solution (SSC) [1] for 20 min at 65, 75, 85, and 100°C, after which the samples were rapidly transferred into a large volume (600 ml) of SSC cooled to 0°C. In the control experiments, incubation was carried out in SSC at 20°C for 20 min. The preparations were quickly removed after 0, 2, 5, 15, 20, 30, 40, 50, and 60 min and transferred into cold (0°C) fixative. At the end of 1 h the fixed preparations were passed through a series of alcohols (absolute ethanol, 96, 60, and 30%) to water and then transferred to buffer and stained in a buffered solution of acridine orange (1.12×10^{-6} M) for 40 min. After staining, the cover slips were placed on slides in a drop of acridine orange solution of the same concentration, and the edges of the slips were mounted with Entellan. Fluorescence of the preparations was excited by an incandescent lamp (maximal intensity of exciting light at $\lambda = 420$ nm). The intensity of fluorescence of the nuclei was measured at $\lambda = 530$ nm with the FÉU-15V photoelectronic multiplier.

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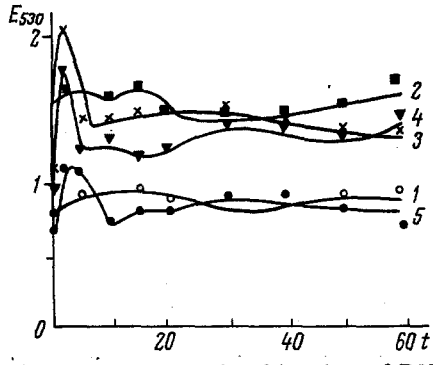

Fig. 1. Renaturation kinetics of DNP complex of cell nuclei denatured by heat during incubation in cold SSC. Abscissa, duration of incubation (in min); ordinate, intensity of fluorescence measured at $\lambda = 530$ nm (E_{530}): 1) cells incubated at 20°C; 2, 3, 4, 5) preparations denatured at 65, 75, 85, and 100°C respectively.

EXPERIMENTAL RESULTS AND DISCUSSION

Renaturation profiles of lymphocytes denatured at various temperatures are shown in Fig. 1. Clearly the renaturation profiles of preparations denatured at 75, 85, and 100°C were similar and consisted of curves with two maxima. During the first 2 min of incubation a sharp increase in the intensity E_{530} was observed with a maximum corresponding to incubation for 2 min. On further incubation a decrease in the intensity of fluorescence was observed, followed by the appearance of a second (local) maximum.

With an increase in the temperature at which the cells were denatured a decrease in the "two-minute" maximum was observed. The values of the first maximum (the difference between the intensities of fluorescence for incubation times of 2 and 0 min, i.e., $E_{530/2\text{min}}-E_{530/0\text{min}}$, in relative units) for preparations denatured at 75, 85, and 100°C were 0.98, 0.76, and 0.39 respectively. The values of the intensity of fluorescence at the first maximum differed significantly for cells denatured at 75, 85, and 100°C ($P_{75-100} < 0.001$; $P_{85-100} < 0.001$; $P_{75-85} < 0.02$). No changes in the intensity of fluorescence during incubation in cold SSC were observed for the control curve. For cells incubated at 65°C the sharp increase in the intensity of fluorescence also was absent, i.e., fluctuations in the intensity of fluorescence during incubation with SSC took place that were not significant, but the curve was shifted along the ordinate toward higher values of E_{530} than the control curve.

The complex character of the dependence of the intensity of fluorescence on the duration of incubation in SSC is explained by the fact that changes taking place both in the protein component of the DNP and also in the DNA make their contributions to the measured intensity of fluorescence. The change in the intensity of fluorescence at E_{530} observed in the present experiments can be explained by two processes taking place at different speeds: renaturation of DNA (as a component of DNP) and conformational changes in DNP taking place with the participation of the protein. The sharp increase in the intensity of fluorescence during the first 2 min of incubation is regarded by the writers as evidence of the high rate of renaturation of DNA as a component of DNP. The second hypothesis seems unlikely to be true.

The high rate of renaturation of DNA during the first 2 min of incubation can be explained by the existence of protein "cross-linkages" that do not permit the denaturing DNA to form long loops. The decrease in the intensity of fluorescence observed on the curves during incubation of the cells for 2-10 min can be explained by the addition of protein to the already renatured DNA. Possibly under these circumstances the lysine-rich fraction, which is known [2] to have greater affinity for native DNA than for denatured, may be added to the DNA. The presence of renaturation of DNA in cells denatured at 75°C shows that denaturation of DNA had already taken place on heating to this temperature. The absence of renaturation on heating to 65°C suggests that no denaturation of DNA takes place at that temperature. Since the renaturation curve of cells heated to 65°C was shifted along the ordinate toward higher values, the presence of reversible dissociation of the proteins can be assumed. The decrease in the intensity of fluorescence in the region of the two-minute maximum with a rise of the temperature at which the cells were denatured can be explained by a relative increase in the proportion of irreversibly denatured DNA.

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