RECORDING MELTING AND RENATURATION PROFILES OF THE DNP COMPLEX OF INDIVIDUAL CELL NUCLEI BY A MICROFLUOROMETRIC METHOD USING ACRIDINE ORANGE

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UDC 612.015.2 :547.963.23].014.43

It was shown by a microfluorometric method that DNA in the DNP individual cell nuclei, when denatured by heat, is capable of rapid renaturation (by 50% after 1 min at 20°C). In the region preceding melting the dye-binding ability of the cells depends on temperature: the maximum of fluorescence (dye-binding) of the nuclei lies within the temperature range 55-65°C. Formaldehyde, in a concentration of 0.2 M, after several minutes substantially weakens the dye-binding properties of the nuclei of hen's erythrocytes and human peripheral blood lymphocytes.

A microfluorometric method of obtaining temperature denaturation (melting) curves of the DNP complex of individual cell nuclei has been described [2, 3]. Since the maximum of fluorescence of acridine orange, when bound with single-helical segments of DNA, lies in the region of 640 nm, and when bound with double-helical segments, in the region of 530 nm it is possible to determine quantitatively the degree of uncoiling or "melting" of DNA as a constituent of the DNP complex from the value of alpha ($\alpha = I_{640}/I_{530}$, where I represents the intensity of fluorescence).

In the investigation described below the effectiveness of the stabilizing action of 4% formaldehyde solution, a concentration regarded as adequate for stabilizing single-helical segments of DNA formed by heating DNA solutions, was studied.

EXPERIMENTAL METHOD

A number of modifications were introduced into the original method of obtaining melting profiles of nuclear DNP [2, 3]: to prevent renaturation of the DNA, the specimens after melting were immediately placed for 30 sec in SSC (salt–sodium citrate solution consisting of 0.15 M NaCl and 0.015 M sodium citrate), the temperature of which was fixed strictly at 0°C. No subsequent dehydration was carried out and the specimens were fixed in a mixture of equal volumes of ethanol and acetone (the fixative) strictly at 0°C. All subsequent procedures were carried out as described in the original method.

Renaturation was studied by the following method. Specimens heated in the melting medium (4% formaldehyde solution in SSC) at 96-97°C for 20 min were quickly transferred to SSC at different temperatures (−3, 0, 8, 20, and 37°C) for 1 min. They were then fixed in the mixture at 0°C, stained, and measured.

The effect of formaldehyde on dye-binding by DNA molecules of the cell nuclei was studied as follows. The fixed specimens were placed in SSC at 20°C, transferred into 0.2 and 4% formaldehyde solution made up in SSC at 0°C, for 1, 10, and 20 min, and then fixed and stained.

Laboratory of General Pathophysiology, Institute of Psychiatry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 12, pp. 42-44, December, 1971. Original article submitted November 24, 1970.

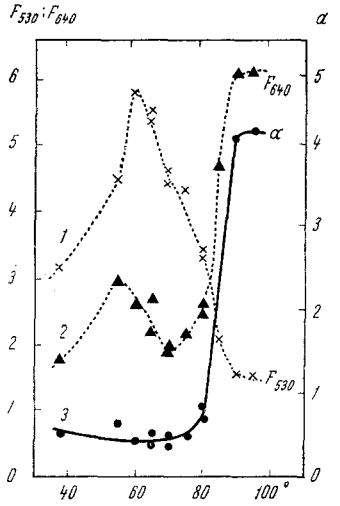

Fig. 1. Change in intensity of fluorescence (in conventional units) and in value of alpha for DNP of individual cell nuclei (human blood lymphocytes) on melting: 1) intensity of fluorescence measured at 530 nm; 2) the same at 640 nm; 3) value of α.

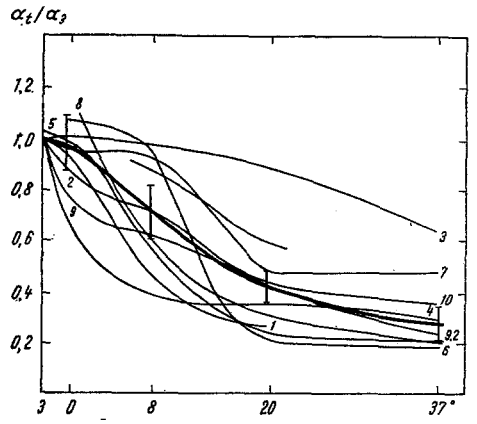

Fig. 2. Effect of temperature on renaturation of DNA of individual cell nuclei. Abscissa, temperature of SSC in which specimens were incubated for 1 min after "melting"; ordinate, ratio between value of α obtained in SSC at a given temperature and value of α obtained in SSE at $-3°C$. Results of individual experiments and combined renaturation curve shown. Absolute values of α observed in control (α_c) and after melting of cells (α_m) also are given.

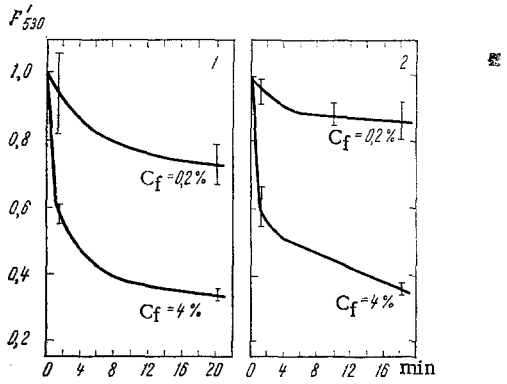

Fig. 3. Effect of formaldehyde on dye-binding ability of DNP of individual cell nuclei. Abscissa, duration of incubation in formaldehyde solutions of different concentrations made up in SSC; ordinate, ratio between intensities of fluorescence of cells incubated in SSC containing and not containing formaldehyde, measured at 530 nm: 1) hens' erythrocytes, 2) human lymphocytes.

Fluorescence of at least ten cell nuclei was measured in each specimen. Light with α = 380-430 nm was used for excitation. An N.A. 1.1 epiobjective, with magnification of 95, was used.

EXPERIMENTAL RESULTS

The value of the coefficient α increased by more than eight times during melting, to reach 4.0 or higher (Fig. 1). This difference between the maximum value of α obtained in the present investigation and when the original method was used (α = 0.6-0.8) cannot be explained by differences in the spectral characteristics of the light receivers used, because the original value of the coefficient α for the intact cells was closely similar.

The low values of the coefficient α obtained by the use of the original method can be assumed to have been due to renaturation of the DNA of the cell nuclei. To test this hypothesis a special investigation of the renaturation process was made. Lymphocytes of human peripheral blood and hen's erythrocytes were used as the test objects.

In work with these objects renaturation of the DNA of preliminarily heated cells was observed to take place fairly rapidly. It is clear from Fig. 2 that incubation of erythrocytes for 20 min in formaldehyde solution did not prevent renaturation of the DNA of the cell nuclei. The rate of renaturation was determined by the temperature of the solution in which the cells were incubated after melting. Incubation of the cells in SSC solution for 1 min at 20°C led on the average to renaturation of 50% of the DNA (or more precisely

to a reduction by half in the value of the coefficient α). The results of ten independent experiments to study renaturation of the DNA of hen's erythrocyte nuclei are illustrated in Fig. 2.

In the region preceding the rise in the value of α (55-65°C), maxima of fluorescence of the cells were present. A study of the relationship between the intensity of fluorescence of the cell nuclei and temperature during heating in medium not containing formaldehyde showed that the occurrence of these maxima cannot be explained by the action of formaldehyde on the specimens: the curves of fluorescence vs. temperature were the same shape as before although the magnitude of their maxima and their position were slightly changed. These changes followed no detectable pattern, but the ability of formaldehyde to change the intensity of fluorescence (the dye-binding properties of the DNP complex) of the cell nuclei was demonstrated by model experiments whose results are given in Fig. 3. Formaldehyde weakened the dye-binding properties of the DNP complex even at 0°C. This action was most marked in the first minutes of the contact between the specimens and the formaldehyde solution.

The results of this investigation showed that the DNA of these cells, when denaturated by heat (96-97°C, 20 min) in a medium containing 4% formaldehyde solution is capable of renaturation. During rapid cooling, renaturation of DNA does not take place in solution, and it is only during slow cooling of DNA denatured by heat that its biological activity is partially restored. The fact that incubation of preliminarily melted cells at 37°C for 1 min leads to a decrease in the value of α almost to the original values evidently means that in this case the chaotic aggregation of DNA is only very slight. The high rate of renaturation observed experimentally can be explained by assuming that the protein of the DNP complex does not allow the complementary DNA strands to move far away from each other, but holds them close together. Under these conditions it is possible that formaldehyde may act predominantly as a weak denaturing agent [1]. The formaldehyde concentration inside the cell nucleus, it must be pointed out, is unknown.

The high rate of DNA renaturation found in the cell nuclei suggests that the supramolecular organization of the DNP complex in the cell nucleus differs substantially from its organization in the corresponding model systems. The existence of special protein "ties" (longitudinal or transverse), fixing the nucleic acid molecules three-dimensionally, may be postulated.

The increase in the intensity of cell fluorescence demonstrated in this investigation in the low-temperature region may be connected with the temperature dissociation of the DNP complex.

LITERATURE CITED

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