CONFORMATIONAL CHANGES IN HEAT-DENATURED NUCLEOPROTEIN COMPLEX OF HUMAN LYMPHOCYTES DURING SUBSEQUENT COOLING

A. R. Stepanyan, R. R. Lideman, and G. B. Zlobina

UDC 612.112.94:612.398.145.1.014.43

To study the kinetics of conformational changes in the nucleoprotein complex (NPC) of human peripheral blood lymphocytes a microfluorometric method of analysis was used, whereby the state of the chromatin could be assessed quantitatively from binding of the dye acridine orange. Extraction of the F_1-fraction of histones with 0.01 N HCl, pH 2.0, essentially modified (delayed) conformational changes in NPC. Extraction of F_1-, F_2-, and F_3-fractions of histones with 0.25 N HCl, pH 0.6, led to more marked delay of the kinetics of the conformational changes. The kinetics of conformational changes of NPC also was delayed when the process was carried out in a more viscous medium (in 70% glycerol solution). In this case the NPC has low values of intensity of fluorescence at 530 nm (F_{530}) and of the ratio I_{640}/I_{530}; this evidently points to irreversibility of denaturation of NPC.

KEY WORDS: lymphocyte nuclei; conformational changes in nucleoprotein complex; microfluorometric method.

One way of studying the nucleoprotein complex (NPC) in cell nuclei is by analysis of its conformational changes after preliminary heating. So far there have been only a few investigations [1, 2, 6, 8] devoted to the analysis of this process. Some of them [1, 2, 8] have revealed very rapid conformational changes in cells heated to 75-100°C and subsequently cooled, which the authors concerned interpreted as rapid renaturation of DNA in the composition of the deoxyribonucleoproteins (DNP) of the cell nuclei. Meanwhile Chamberlain and Walker [6] found no renaturation of DNA in the composition of DNP in cell nuclei heated to high temperatures in a viscous medium and then cooled.

To continue the study of this problem the next step was to examine the kinetics of conformational changes associated with different degrees of deproteinization of NPC and in media of different viscosity, for it is the protein component of NPC that is regarded as the factor responsible for rapid renaturation of DNA in the DNP of the cell nuclei. In particular, it is claimed that protein of the DNP complex does not permit the complementary chains of DNA to move apart through any considerable distance, but retains them in the immediate vicinity of one another and so facilitates renaturation of DNA.

EXPERIMENTAL METHOD

Peripheral blood lymphocytes from 14 healthy blood donors were used. Cultures of intact white blood cells obtained by the method of Rigler and Killander [9] were fixed in ethanol-acetone (1:1) fixative for 1 h.

To deproteinize the NPC acid extraction of basic proteins from the fixed intact cells was carried out [3]. Preparations were taken through a series of alcohols from the fixative into an aqueous medium, and some specimens were incubated in water (control), the rest in 0.01 and 0.25 N HCl, pH 2.0. Extraction continued for 20 min at 0°C. After incubation in acid, the preparations were washed in citrate-phosphate buffer, pH 4.1, at 0°C for 3 min and fixed for 1 h. In the investigation by Matveeva and Lideman [3] electrophoretic control of the extraction showed that under these conditions 0.01 N HCl at pH 2.0 extracts mainly the F_1, whereas 0.25 N HCl at pH 0.6 extracts the F_1-, F_2-, and F_3-fractions of histones.

To study the kinetics of renaturation the cells were heated to 75-80°C, using the procedure described earlier [1]. When a viscous medium was used, the fixed cells were incubated in a solution containing 70% glycerol and 30% citrate-salt solution (SSC) and not in SSC alone, for 20 min at 75°C, after which the holder

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. 88, No. 12, pp. 680-683, December, 1979. Original article submitted October 15, 1978.

 0007-4888/79/8812-1428$07.50

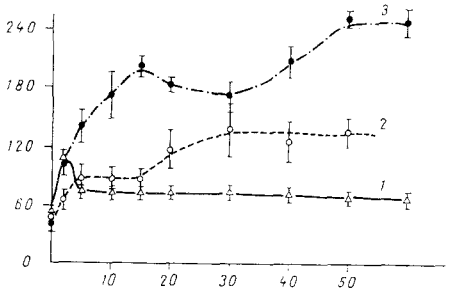

Fig. 1

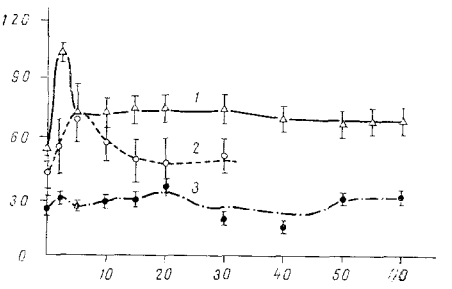

Fig. 2

Fig. 1. Kinetics of conformational changes of heat-denatured NPC from lymphocyte nuclei during incubation in cold SSC. 1) Native (nondeproteinized) NPC; 2) NPC treated with 0.01 N HCl; 3) NPC treated with 0.25 N HCl. Here and in Fig. 2: abscissa, incubation time (in min); ordinate, intensity of fluorescence at 530 nm.

Fig. 2. Kinetics of conformational changes of heat-denatured NPC from lymphocyte nuclei during incubation in cold SSC and in 70% glycerol solution. 1) Native (nondeproteinized) NPC incubated in SSC; 2) native NPC incubated in 70% glycerol solution; 3) deproteinized (0.25 N HCl) NPC in 70% glycerol solution.

with the slides was quickly transferred into a large volume (600 ml) of a solution containing 70% glycerol and 30% SSC cooled to 0°C. The subsequent procedures were the same as those described earlier [1]. The cells were stained in buffered 1.12×10^{-6}M solution of acridine orange. To estimate the degree of spiralization the coefficient α ($\alpha = I_{640}/I_{530}$) was used, where I is the intensity of fluorescence measured at 640 and 530 nm. Since the values of F_{530} (the intensity of fluorescence of the lymphocyte nuclei) are low when acridine orange is used in a concentration of 1.12×10^{-6}M, in order to measure the coefficient α the dye was used in a concentration of 1×10^{-5}M.

EXPERIMENTAL RESULTS

The kinetics of conformational changes of the deproteinized NPC of human peripheral blood lymphocytes, previously heated to 80°C, was studied during subsequent incubation in cold SSC. Dependence of the intensity of fluorescence of the lymphocyte nuclei (F_{530}) on the duration of incubation (t) in cold SSC is shown in Fig. 1 for cells deproteinized with 0.01 N HCl (curve 2) and with 0.25 N HCl (curve 3). For comparison, the analogous curve 1 for native (nondeproteinized) NPC is also shown in Fig. 1. Clearly a rapid kinetics of F_{530} with a maximum at $t = 2$ min was discovered for the native NPC, in agreement with results obtained by Artyushin et al. [1]. It will also be seen that extraction of histone fraction F_1 with 0.01 N HCl, pH 2.0, significantly modified the kinetics of conformational changes in the NPC, leading to flattening out of the F_{530} values on a plateau at $t = 5$ min. The difference between the values of F_{530} at $t = 5$ and $t = 0$ min in relative units was 44 ± 16 ($P < 0.02$). During incubation for 15-20 min a further increase in F_{530} was observed, with flattening out on a plateau after incubation for 20-50 min. The difference between the values of F_{530} for cells at $t = 50$ and $t = 0$ min was 91 ± 25 ($P < 0.002$). During extraction of histone fractions F_1, F_2, and F_3 (Fig. 1, curve 3) with 0.25 N HCl at pH 0.6, a more marked slowing of the kinetics of conformational changes in NPC was observed (flattening out on a plateau at $t = 15$ min). The difference between the values of F_{530} at $t = 15$ and $t = 5$ min was 75 ± 15 ($P < 0.001$). During incubation for 30-50 min a further increase in F_{530} was observed, with the appearance of a second plateau corresponding to an incubation time of 50-60 min. The difference between the values of F_{530} at $t = 50$ and $t = 15$ min was 46 ± 11 ($P < 0.001$).

Conformational changes in the native (nondeproteinized) NPC in a viscous medium (70% glycerol solution instead of SSC) had the appearance shown in Fig. 2, curve 2. For comparison, the accompanying curve 1 in Fig. 2 shows the kinetics of conformational changes of NPC in SSC. It will be seen that when the process takes place in a more viscous medium, some slowing of the conformational changes is observed: the highest value of F_{530} was observed at $t = 6 \pm 1$ min ($P < 0.001$), but the shape of the curve reflecting the dependence of F_{530} on time was unchanged.

The kinetics of conformational changes of the deproteinized NPC (histone fractions F_1, F_2, and F_3 were extracted with 0.25 N HCl) in a viscous medium (70% glycerol solution) is shown in Fig. 2, curve 3. Clearly

under these conditions no conformational changes could be detected in the NPC. The values of F_{530} compared with the control (i.e., with cells from which the histones had been extracted with 0.25 N HCl and, not having been heated, were fixed, after which F_{530} was measured) were relatively small under these conditions. The value of F_{530} for NPC in the control was 84 ± 16, whereas for deproteinized NPC in a viscous medium at t = 0 min it was 25 ± 1, i.e., there was a more than threefold decrease in F_{530}. The difference between these values of F_{530} was 59 ± 16 ($P < 0.002$), and the difference between the values of F_{530} in the control and at t = 60 min was 51 ± 16 ($P < 0.01$). It was also shown that under these conditions the coefficient α was increased. For control cells it was 61 ± 7, and for the heated cells at t = 0 min it was 125 ± 8 and at t = 50 min 103 ± 9, i.e., a twofold increase in the coefficient α was observed, indicating denaturation of NPC.

In the modern view [4, 5, 7], F_1, bound with the complex of DNA and the F_{2a}-, F_{2b}-, F_3-, and F_4-fractions of histones, plays an important role in the formation of the supramolecular organization (condensation) of NPC. In this connection, the significant slowing of conformational changes during extraction of the F_1-fraction of histones by 0.01 N HCl, observed in the present investigation, suggests that rapid conformational changes are determined by the supramolecular organization of NPC. Extraction of histone fractions F_1, F_2, and F_3 by 0.25 N HCl leads to more marked slowing of conformational changes. Conformational changes also are slowed if the process takes place in a viscous medium (70% glycerol solution). No conformational changes were found for deproteinized NPC in a viscous medium. In this case NPC had low values of F_{530} and higher values of the coefficient α, evidently indicating that irreversible denaturation of the NP complex takes place under these conditions.

LITERATURE CITED

1. S. K. Artyushin, R. R. Lideman, and G. P. Zlobina, Byull. Éksp. Biol. Med., No. 1, 44 (1975).
2. G. P. Zlobina, Byull. Éksp. Biol. Med., No. 12, 42 (1971).
3. N. P. Matveeva and R. R. Lideman, Tsitologiya, 17, 432 (1975).
4. J. Bonner, in: Cell Differentiation in Microorganisms, Plants and Animals, Jena (1977), p. 76.
5. K. Brash, Exp. Cell Res., 101, 396 (1976).
6. P. I. Chamberlain and P. M. B. Walker, J. Mol. Biol., 11, 1 (1965).
7. A. J. Louie and J. H. Dixon, Nature New Biol., 243, 164 (1973).
8. D. Nash and W. Plaut, Proc. Natl. Acad. Sci. USA, 51, 731 (1964).
9. R. Rigler and D. Killander, Exp. Cell Res., 54, 171 (1969).