TOPOLOGIC HETEROGENEITY OF DNA IN NUCLEOIDS OF HUMAN LEUKOCYTES

V. D. Paponov, S. P. Rad'ko, and E. G. Shcheglova

UDC 612.112.014.24:577.212.3+616.155.392-07:616.155.3-008.9:577.212.3

KEY WORDS: DNA, heterogeneity, leukocytes

Deproteinized nuclei of eukaryotic cells (nucleoids), obtained in cellulolytic solutions but preserving their spherical nuclear skeletons with all their nuclear DNA, have attracted the attention of research workers because they can be used as model systems of the topologic (supercoiled) organization of genomic DNA of living cells. Analysis of the DNA topology in nucleoids is usually carried out by titration of DNA with intercalating agents, leading to transformation of negative supercoiled molecules into positive in domains of circular supercoiled DNA (cscDNA). Structural transition is manifested as extremal dependence of the hydrodynamic characteristics of nucleoids on concentration of the intercalating agent. The titration curves have a minimum on sedimentation analysis [6] and a maximum on rheologic analysis [2].

The position of the single extremum on the titration curve permits evaluation only of an average degree of supercoiling of nuclear DNA. However, data on the effect of supercoiling of the circular DNA of phages and plasmids on transcription in vitro [5, 10] and in vivo in *Xenopus laevis* oocytes [8] respectively have led to the natural idea that the DNA in circular domains of eukaryotic genomes exhibits topologic heterogeneity as a reflection of the regulation of gene expression at this level. In recent years the presence of two [4] or even three minima has been reported on sedimentation titration curves of eukaryotic cell nucleoids with ethidium bromide (EtBr). However, the authors explained their data without involving the idea of topologic heterogeneity of nucleoid DNA, mainly because of the small number of maxima.

In the investigation on human leukocytes described below it is shown that rheologic (viscosimetric) titration of nucleoids with ethidium bromide makes it possible to obtain at least eight maxima, whose position on the titration curve differs in the case of leukocytes from patients with different types of leukemia. Thus investigations of the genome during cell differentiation, aging, and various pathological states, acquire a very rewarding prospect.

EXPERIMENTAL METHOD

The procedures of taking venous blood from healthy donors and patients and isolating the leukocytes were described previously [3]. A dispersion of leukocytes in 0.15 M NaCl + 0.7 mM Na-phosphate buffer (pH 7.0) was converted into lytic medium by mixing it with an equal volume of a solution of 2 M NaCl + 0.2 M Na_2EDTA + 4 mM Tris + 1% Triton X-100 + 2 mM PMSF (phenylmethylsulfonyl fluoride), and the pH was adjusted to 8.0 with NaOH. The viscosity of the resulting dispersion of nucleoids in the presence of different concentrations of EtBr was measured at 25°C on a rotary viscosimeter with a shear stress of $4.5 \cdot 10^{-3}$ dyne/cm^2. The accuracy of measurement of viscosity was ±0.1%. Concentrations of nucleoids of leukocytes from different individuals were equalized before the measurements were made on the basis of the approximate equality of viscosity of their dispersions in the absence of EtBr.

EXPERIMENTAL RESULTS

Dependence of the relative viscosity of a nucleoid dispersion from peripheral blood leukocytes of a healthy blood donor (Fig. 1a, 1) and from a patient with leukemia (Fig. 1a,

Institute of Medical Genetics, Academy of Medical Sciences of the USSR. Kazan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 194-195, February, 1988. Original article submitted June 2, 1987.

0007-4888/88/0002-0225$12.50

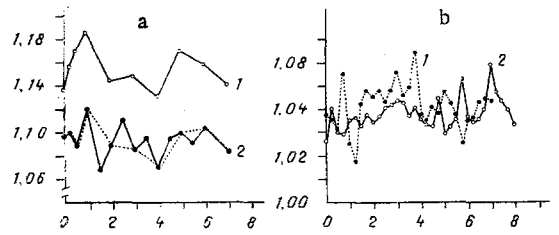

Fig. 1. Dependence of relative viscosity of leukocytes in lytic medium on EtBr concentration. Abscissa, EtBr concentration (in μg/ml); ordinate, relative viscosity η of solution/η of solvent. a:1) Healthy human leukocytes; 2) leukocytes from patient with acute leukemia; b:1) leukocytes from patient with acute leukemia; 2) leukocytes from patient with chronic lymphatic leukemia.

2) in lytic medium on the concentration of added EtBr is shown in Fig. 1a. Curve 1 was obtained by analysis of mixtures of nucleoids with EtBr, differing by 1 μg/ml in their EtBr concentration. Curve 2 was obtained by the use of mixtures differing in their EtBr concentration by 0.5 μg/ml. The broken line shows the appearance of curve 2 which it would have if the method of analysis of the effect of EtBr on nucleoid viscosity was the same as when curve 1 was obtained. Clearly, under the same conditions of analysis, curves for leukocyte nucleoids from the healthy blood donor and the patient with leukemia were very similar. The positions of the first maxima along the abscissa (1 μg/ml) and of the second small maxima (3 μg/ml) coincide. The third maximum on curve 1 corresponds to an EtBr concentration (C_{EtBr}) of 5 μg/ml, whereas on curve 2 C_{EtBr} = 6 μg/ml.

It is interesting to note that during sedimentation analysis of the effect of EtBr on nucleoids of peripheral blood lymphocytes from a healthy blood donor and a patient with leukemia, and having obtained a single extremum (minimum) on the titration curves, Hartwig [9] found that in the case of the patient's nucleoids this minimum was further to the right along the abscissa. This suggested a greater degree of supercoiling of DNA in nucleoids of the patient's lymphocytes. Comparison of the position of the maxima in Fig. 1a suggests that there are three groups of domains in nucleoids of healthy and leukemic human leukocytes which differ in their degree of supercoiling, which in the patient was higher only for the third group of domains with the highest density of supercoiling. However, different interpretations of the nucleoid titration curve with 2 and 3 extrema are possible.

During sedimentation analysis of the effect of EtBr on mouse thymocyte nucleoids Filippovich et al. [4] found two minima, but connected their presence with differences in the accessibility of cscDNA domains for EtBr in a heterogeneous system of cell lysates. However, during a similar analysis of homogeneous solutions of deproteinized circular DNA of virus SV40 and phage PM2, two minima also were discovered on the EtBr titration curves [7]. It has been shown [11] that the EtBr sedimentation titration curve of circular DNA of phage λ has one minimum in medium of low ionic strength, but in 1 M NaCl, this principal minimum is joined by two additional shallow minima on the right and left of the titration. On the basis of these data, Luchnik [1] concluded that the three minima which he found on the EtBr sedimentation titration curve of murine erythroleukemia cell nucleoids reflect the properties of the supercoiled DNA and are not the result of heterogeneity of the nucleoids. The first and third minima were explained by A → B and B → C conformational transitions, respectively, whereas the second (principal) minimum was explained by conversion of a negative supercoiled molecule into positive [1].

It will be clear from Fig. 1a that rheologic analysis of the effects of EtBr on human leukocyte nucleoids can reveal 5 maxima of viscosity if the mixtures of cell lysates with EtBr differ in EtBr concentration by 0.5 μg/ml. If, however, these mixtures differed by C_{EtBr} = 0.25 μg/ml, it was now possible to detect eight maxima of viscosity on human leukocyte nucleoid titration curves within the EtBr concentration range from 0 to 8 μg/ml (Fig. 1b). The small maxima may arise through conformational transitions between known forms of DNA (A, B, C, Z) and also between them and cross-shaped structures on local regions of cscDNA. The large maxima reflect structural transitions modifying the tertiary structure of the nucleoid DNA more profoundly, i.e., transitions from a negative to a positive helix. Instead

of one large extremum, described by other workers previously [1, 2, 6, 7, 9, 11], by a more fractional titration we found several large maxima of roughly equal value. In this connection the presence of groups of cscDNA domains with different densities of supercoiling in nucleotides may be suggested. Thus the titration curves illustrated in Fig. 1b are evidence of topologic heterogeneity of DNA in human leukocyte nucleoids.

Differences in the leukocyte nucleoid titration curves for leukemia patients in Fig. 1b may prove useful for the diagnosis and analysis of the pathogenesis of leukemias and also, perhaps, of other types of pathology connected with disturbances of the genetic apparatus of cells.

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SENSITIVITY OF SPLEEN CELLS OF DIFFERENT STRAINS OF MICE TO THE ANTIPROLIFERATIVE ACTION OF ALKYLATING COMPOUNDS

A. L. Pukhal'skii, A. P. Mezhneva, and L. A. Pevnitskii

UDC 612.411.014.2:612.6].014.46:615.277.3

KEY WORDS: antiproliferative action, chlorbutin, maphosphamide, cyclophosphamide, mouse genotype

Differences in the sensitivity of mice of different lines to the immunodepressive action of alkylating agents depend on a number of genetically controlled factors. To analyze such a multifactorial system, its individual components must be distinguished so that the contribution of each of them to the general pattern observed in vivo can subsequently be determined.

The aims of the present investigation were to develop a quantitative method of assessment of the degree of sensitivity of lymphoid cells to the antiproliferative action of alkylating preparations and to study the effects of different alkylating compounds on proliferation of spleen cells of mice of different genotypes.

EXPERIMENTAL METHOD

Male mice weighing 20-24 g of the following inbred lines were used: BALB/cJLacSto, DBA/2 JSto, CC57Br/MVRap, C57Bl/6JSto. The animals were killed by cervical dislocation and the spleens were removed under sterile conditions, and transferred into a glass homogenizer to

Laboratory of Immunogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 196-198, February, 1988. Original article submitted May 27, 1987.