BROMODEOXYURIDINE-INDUCED RELAXATION OF DNA–PROTEIN INTERACTIONS IN CHROMATIN AS REVEALED BY NUCLEOPROTEIN-CELITE CHROMOTOGRAPHY

M. M. Zaboikin, S. L. Kolobkov,
N. I. S'yakste, A. V. Likhtenshtein,
and V. S. Shapot

KEY WORDS: Rauscher's erythroleukemia; bromodeoxyuridine; chromatin; DNA–protein interaction; nuclear RNA.

Bromodeoxyuridine (BDUR), a thymidine analog, has the unique property, when incorporated into DNA of differentiated cells, of selectively inhibiting their specialized functions without significantly affecting their viability [5, 6, 12]. Although some tumor cells still continue to proliferate actively after incorporating BDUR, they lose their ability to induce tumors in animals. This property is restored, however, as the BDUR is eliminated from the DNA [12]. The elucidation of the molecular mechanisms of action of BDUR is evidently of great importance also to the understanding of how gene activity is regulated in normal cells, and to detect its particular features in tumor cells. The view is generally held that BDUR-substituted DNA forms more stable complexes than intact DNA with histones and with nonhistone proteins, as a result of which the mosaic of active and inactive regions of the genome, characteristic of normal cells, is modified [7, 10, 13]. These views are based largely on the results of model experiments in which artificially formed complexes of DNA (intact and BDUR-substituted) with definite fractions of nuclear proteins were compared for stability in solutions of increasing ionic strength [10, 13].

In the present investigation an attempt was made to test the hypothesis that BDUR can strengthen DNA–protein interaction in chromatin, using the technique of nucleoprotein-celite chromatography (NPC-chromatography), suggested by the writers recently, by means of which the stability (resistance ot dissociation) of nucleic acid–protein interactions can be tested [1, 2]. In this method, proteins of nucleoproteins are absorbed irreversibly on celite, and nucleic acids of various types are successively released from binding with protein and are fractionated in accordance with the strength of these bonds. Since nucleic acids in the composition of unfractionated cell digests can be analyzed by this method [2], an attempt also was made to study changes induced by BDUR, not in artificial complexes, but in intact chromatin, without preliminary isolation, a procedure fraught with various artifacts.

EXPERIMENTAL METHOD

A suspension culture of Rauscher's erythroleukemia cells in the logarithmic phase of growth was used. The cells were labeled with thymidine-^{14}C (2 μCi/ml) for 4 h and with thymidine-^{3}H (7.5 μCi/ml) for 4 h, after which the cells were preincubated with BDUR (20 μg/ml) for 15 h. Since the generation time of the cells studied is approximately 22 h, under the above conditions BDUR is incorporated into only one of the two DNA strands (Fig. 1).

Heterogeneous nuclear RNA of intact cells and cells incubated with BDUR were labeled with uridine-^{3}H (50 μCi/ml) for 20 min in Eagle's medium, having been kept beforehand for 60 min with actinomycin D (0.05 μg/ml) to produce selective inhibition of rRNA synthesis. Gradient elution of nucleic acids from the nucleoprotein-celite column was carried out as described previously [2], using unfractionated cell digests as original material. The degree of substitution of thymine by BDUR was calculated from the data of centrifugation of purified DNA in a CsCl gradient by the equation in [7].

Laboratory of Biochemistry of Tumors, Onocologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 4, pp. 420-422, April, 1980. Original article submitted October 30, 1979.

0007-4888/80/8904-0439$07.50

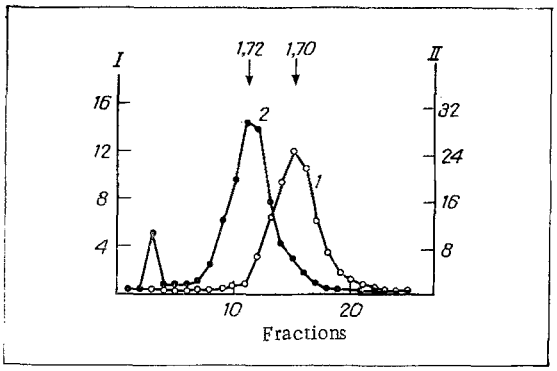

Fig. 1. Distribution of intact and BDUR-substituted DNA in a cesium chloride density gradient: 1) intact DNA-^{14}C; 2) BDUR-substituted DNA-^{3}H. Arrows indicate buoyant density of DNA studied (in g/cm^3). Ordinate: I) CPM×10^3 for 3 h, II) CPM×10^{-2} for ^{14}C.

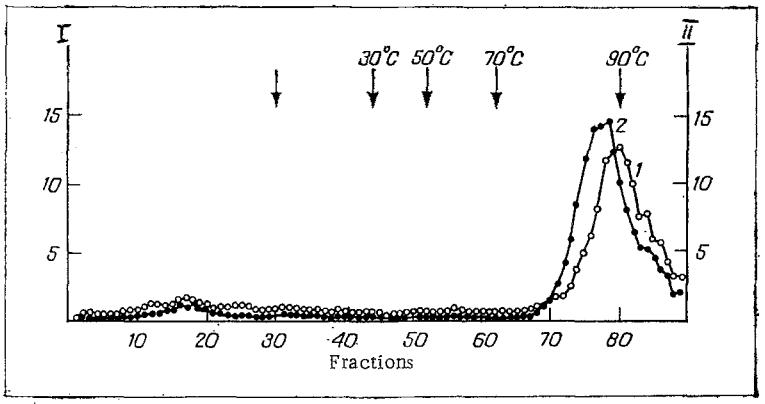

Fig. 2. MPC-chromatography of intact and BDUR-substituted DNA. Arrows indicate beginning and continuation of temperature gradient. Remainder of legend as in Fig. 1.

EXPERIMENTAL RESULTS

The density distribution of molecules of double-labeled (with carbon and tritium, respectively) intact and BDUR-substituted DNA in a CsCl equilibrium gradient is illustrated in Fig. 1. The increase in the density of DNA as a result of incubation of the cells for 15 h with BDUR was equivalent to 20% substitution of thymine calculated as total DNA.

Replacement of thymine by BDUR was shown to modify the strength of DNA–protein interactions in the chromatin significantly. As Fig. 2 shows, the chromatographic position of BDUR-substituted DNA was shifted compared with the intact DNA, but in the diametrically opposite direction to that expected on the grounds of data already published [5, 6, 9]. It was found that to liberate BDUR-substituted DNA from binding with proteins, a temperature of roughly 2-5°C lower than that required for elution of intact DNA (about 90°C) was required. Judging from the chromatographic test used, BDUR thus does not strengthen but, on the contrary, relaxes DNA–protein interaction in chromatin.

The unexpectedly discovered relaxing effect of BDUR can relate only to the strongest nucleic acid–protein interaction, for in such high concentrations of salt and urea as were used in the course of MPC-chromatography, the bonds between all histones and the majority of nonhistone proteins on the one hand, and DNA on the other hand, should have been broken actually during elution by the salt–urea gradient, before the beginning of the temperature gradient.

Incorporation of BDUR into DNA evidently also affects RNA synthesis. A mixture of intact cells and cells incubated with BDUR, labeled with uridine-^{14}C and uridine-^{3}H, respectively, was subjected to NPC-

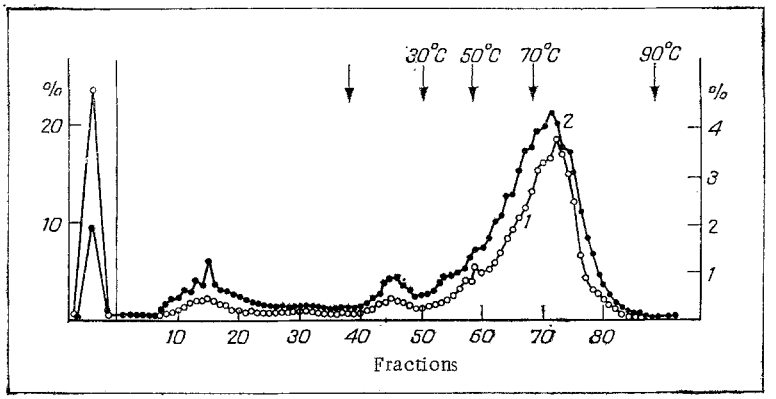

Fig. 3. MPC-chromatography of rapidly labeled heterogeneous nuclear hnRNA in intact cells and in cells incubated beforehand with BDUR. 1) hnRNA-^{14}C of intact cells; 2) hnRNA-^{3}H of cells incubated with BDUR. Fractions 1-3 contain RNA not bound with the column. Ordinate, radioactivity of fraction (in % of total radioactivity of the given isotope applied to the column): scale on left for first three fractions, scale on right for all the rest. Remainder of legend as in Fig. 2.

chromatography. As Fig. 3 shows, the heterogeneous RNA were separated by this method into several fractions [2]: a) low-molecular-weight RNA with rapid turnover, not bound with the column, i.e., not associated or only very weakly associated with proteins (fractions 1-3); b) RNA "intermediate" as regards binding with protein and eluted by a salt–urea gradient (fractions 8-20); c) RNA firmly bound with protein and eluted by the temperature gradient (fractions 40-80). As was shown previously [2], RNA of the two last groups of fractions differed significantly in size. Incorporation of the radioactive label into fractions of nuclear RNA of cells treated with BDUR was found to differ from that of intact cells: Incorporation of label into the first fraction was sharply inhibited, whereas into the other two, on the contrary, it was stimulated a little.

The results thus indicate that the biological activity of the BDUR may be based on the relaxation which it induces in DNA–protein bonds in intact chromatin, and that this modification may be reflected variously in the rate of synthesis of different fractions of heterogeneous nuclear RNA.

LITERATURE CITED

1. A. V. Likhtenshtein, M. M. Zaboikin, V. L. Moiseev, et al., Dokl. Akad. Nauk SSSR, 245, 1005 (1979).
2. A. V. Likhtenshtein (A. V. Lichtenstein), R. P. Alechina, and V. S. Shapot, Biochem. J., 147, 447 (1975).
3. D. A. Colbert and J. R. Coleman, Exp. Cell Res., 109, 31 (1977).
4. S. Githens, R. Pictet, P. Phelps, et al., J. Cell Biol., 71, 341 (1976).
5. J. S. Gordon, G. I. Bell, H. C. Martinson, et al., Biochemistry (Washington), 15, 4778 (1976).
6. S.-Y. Lin, D. Lin, and A. D. Riggs, Nucleic Acids Res., 3, 2183 (1976).
7. D. C. Luk and M. D. Bick, Anal. Biochem., 77, 346 (1977).
8. P. M. Price, Biochim. Biophys. Acta, 447, 304 (1976).
9. S. A. Schwartz, Biochemistry (Washington), 16, 4101 (1977).