MODIFICATION OF STRUCTURAL TRANSITIONS OF SUPRAMOLECULAR NUCLEOPROTEIN SYSTEMS BY THE PROTEIN COMPONENT OF DEOXYRIBONUCLEOPROTEIN FROM MALIGNANT CELLS

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The possibility of dissociation of an unidentified factor responsible for differences in the thermomechanical parameters of supramolecular DNP systems from normal and malignant tissues was demonstrated. The transfer of this factor to DNP from normal tissues leads to transformation of the thermomechanical parameters of the DNP to those characteristic of DNP from malignant tissues. Sonication (10 kHz) for more than 2 min leads to loss of activity of this factor, indicating its macromolecular nature. It is concluded from the results of ultracentrifugation and sonication and also of spectrophotometry that DNA is not a component of this factor.

The search for specific differences between malignant and normal tissues is one of the most important problems in theoretical medicine. Systems concentrating the hereditary information are particularly interesting in this connection because the property of malignancy arising in a cell is transmitted by heredity to subsequent generations of cells. However, the overwhelming majority of investigators at the present time consider that the physicochemical state of the DNA of various human and animal tumors is unchanged during neoplastic degeneration [10-12, 15, 16, 18, 20]. In the few investigations in which the deoxyribonucleoproteins (DNP's) of normal and malignant tissues were compared, no significant differences likewise were found [13, 14, 21].

Most of these investigations have been carried out on solutions of DNA and DNP. However, DNP in the cell is in a medium with physiological ionic strength in which this compound is insoluble, i.e., it is a phase-bounded supramolecular system. Investigation of supramolecular DNP systems has yielded results indicating a significant difference between the properties of these objects when isolated from normal and malignant cells [8]. This difference is expressed as a change in the amplitude of melting of the supramolecular systems within the zone of helix-coil transition of DNA. Meanwhile no such relationship is found in DNA macromolecules themselves when isolated from these DNPs. This suggests that the differences observed are connected with the effect of the protein component of the nucleoprotein complex. The possible mechanisms of this effect have been discussed previously [6].

In the investigation described below an attempt was made to isolate this component and then to analyze its effect on the properties of DNA and DNP obtained from normal tissues.

EXPERIMENTAL METHOD

Noninbred albino rats weighing 150-200 g were used. The tumor, an ascites form of Zajdela's hepatoma (AZH), was taken on the 6th day after intraperitoneal inoculation. Nuclei were isolated from the liver of normal rats and from ascites cells by the method of Allfrey et al. [9]. The nuclei were washed with hypotonic solution and then centrifuged at 1,500 g to remove blood. DNP was isolated from the nuclei by the

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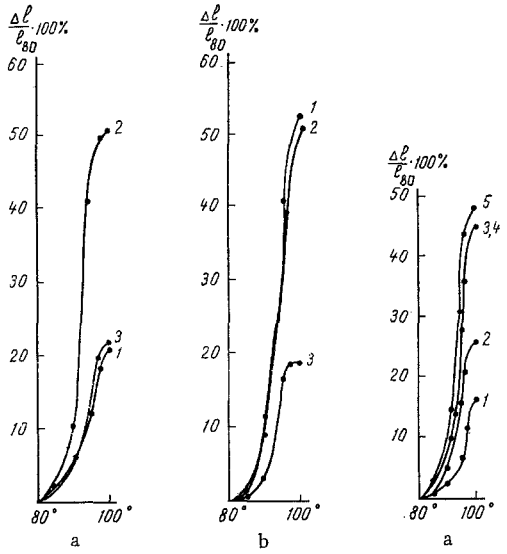

Fig. 1

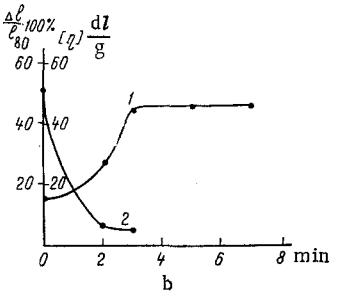

Fig. 2

Fig. 1. Relative contraction of fibers in the region of the helix-coil transition of DNA. a:1) DNP from AZH, N/P = 4.2; 2) DNP from liver, N/P = 4.4; 3) reconstructed DNP preparation from AZH (medium 0.14 M NaCl solution, pH 6.9). $\Delta l/l_{80} = (l_{80} - l_t)/l_{80}$, where l_{80} is the length of the fiber at 80° C and l_t its length at the corresponding temperature. b:1) DNP from liver, N/P = 4; 2) DNP from liver, N/P = 4.4; 3) DNP complex from liver + protein fraction of DNP from AZH, N/P = 4.4 (medium 0.14 M NaCl, pH 6.9).

Fig. 2. Curves of relative contraction of DNP fibers in the region of the helix-coil transition of DNA. a:1) DNP from AZH, N/P = 4.4; 2) DNP from AZH (sonication for 2 min) + DNP from thymus, N/P = 4.4; 3) DNP from AZH (sonication 3 min) + DNP from thymus; 4) DNP from calf thymus, N/P = 4; 5) DNP from thymus (sonication 2 min) + native DNP from thymus (preparation mixed in the ratio 1 : 1 by DNA concentration; medium 0.14 M NaCl solution, pH 7.9). b : 1) relative contraction of DNP fiber in region of helix-coil transition of DNA as a function of sonication; 2) change in coefficient of viscosity $[\eta]$ of DNP depending on duration of sonication.

method of Mirsky and Pollister [19] in Kulikova's modification [1]. The preparations were clarified by centrifugation at 10,000 g for 30 min and the following determinations were made: the concentrations of DNA [3] and protein [17], the molar coefficient of extinction (E_p), and the coefficient of viscosity (η) in a low-gradient viscosimeter with gradients of $\beta = 53.7$, 37.3, and 26.6 sec^{-1} relative to water [4]. Oriented supramolecular systems were obtained and investigated by the method described previously [2, 7].

The specimens were subjected to sonication on a magnetostriction apparatus (10 kHz), and investigated with the SPU-1M spectropolarimeter. The optical density of the specimens tested did not exceed 0.380 in air. Ultracentrifugation was carried out at 200,000 g for 6 h on the MOM-3170 apparatus.

EXPERIMENTAL RESULTS

The components of the DNP complex were separated by dissociation of the DNP at an ionic strength of $\mu = 2.6$. It was hoped to discover whether the action of the factor responsible for the characteristic behavior of supramolecular DNP systems from the tumor is lost after dissociation and reassociation of the DNP complex. For this purpose, the ionic strength of the medium was increased to $\mu = 2.6$, the preparation was stirred for 30 min, and then dialyzed for 18 h against 100 volumes of 0.7 M NaCl. DNP fibers were obtained from the DNP solution obtained in this manner at $\mu = 0.14$ and investigated. As the results given in Fig. 1a show, the characteristic properties of the DNP systems from the hepatoma were completely restored.

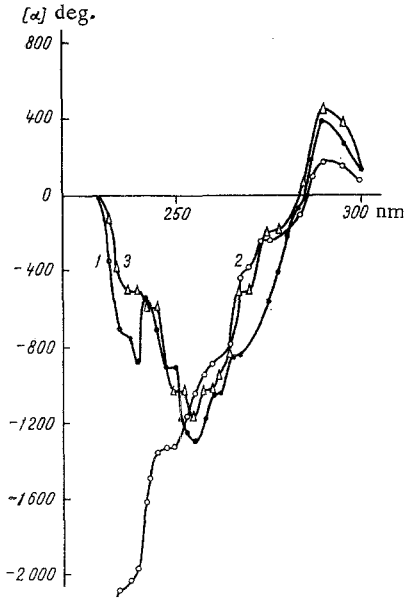

Fig. 3. Curves of dispersion of optical rotation: 1) DNP from liver, N/P = 3.7; 2) DNP from AZH, N/P = 4.1; 3) DNP from thymus, N/P = 4.0 (medium 0.7 M NaCl solution, pH 6.9).

TABLE 1. Changes in Coefficient of Vissity and EP Depending on Duration of Sonication

Duration of sonication (in min)	[η] (in dl/g)	Ep
0	52	6 700
2	6	6 750
3	5	6 750
5		6 800

An attempt was then made to rule out any possible effect of high-polymer tumor DNA on the behavior of the DNP systems from normal tissues. For this purpose the dissociated DNP complex was treated by sonication (concentration 0.3 mg DNA/ml; Table 1).

The sonicated DNP preparations from the hepatoma were mixed with DNP from calf thymus ($\mu = 2.6$) in the ratio 1:1, and after stirring and adjustment by dialysis to $\mu = 0.7$, the rheological parameters of the supramolecular DNP systems ($\mu = 0.14$) were investigated. In a parallel control series, calf thymus DNP was sonicated, mixed with the native preparation, and after dialysis in the same way the corresponding characteristics of the DNP systems also were analyzed ($\mu = 0.14$).

It will be clear from Fig. 2a that if sonication of the tumor DNP did not exceed 2 min in duration, after its addition to DNP from the thymus the amplitude of the helix-coil transition was 26%, whereas the native preparation from calf thymus with the same N/P value gave an amplitude of transition of 45-50% [7].

These results show that the DNA component of the DNP complex is not responsible for the characteristic behavior of the DNP systems from the tumor. However, it will be noted that the amplitude of the helix-coil transition of the corresponding complexes increased with an increase in the duration of sonication (Fig. 2a, b), thus indicating the macromolecular nature of the factor responsible for this effect. Finally, ultracentrifugation of the dissociated DNP ($\mu = 2.6$) from the malignant cells resulted in the virtually complete removal of the DNA components. The supernatant accounted for about 60% of the protein of the original complex. Its addition to DNP from the thymus or liver led to a threefold decrease in the amplitude of the helix-coil transition (Fig. 1b).

The results described above thus indicate that the DNP complex from malignant tissues contains a certain active non-deoxyribonuclear principle of macromolecular nature which leads to a resultant decrease in the amplitude of melting of the condensed DNP systems. The possibility that this factor can be isolated and that it can influence DNP from normal tissues leads to the hope that it will be identified chemically.

It was pointed out above that investigations of the physicochemical characteristics of DNP solutions have revealed no differences in the properties of normal and tumor DNPs or of their DNA components. It was therefore interesting to discover whether DNP macromolecules have any special features in solution, and for this purpose the method of spectropolarimetry was used, for in this method the molecular parameters both of the DNA and of the nonnuclear component of the DNP complex can be analyzed. The curves of dispersion of optical rotation (DOR) of DNP's isolated from calf thymus, rat liver, and AZH, obtained in this way are shown in Fig. 3. It will be seen that in the case of DNPs isolated from normal cells the DOR curve has the characteristic shape with a minimum of rotation at 255 nm. The DOR curves for DNP and AZH have differences and, in particular, the maximum at 255 nm is absent.

Conjecturally the cause of the abnormal behavior of DNP from tumor tissues is due to increased coiling of their protein components or to an increase in the number or length of the zones of unstabilized DNA. The existence of a definite causal connection between these two phenomena cannot be ruled out. It is important to note that a fairly low amplitude of melting of the supramolecular DNP systems is also

characteristic of condensed chromatin which the writers isolated from liver chromatin [2]. However, whereas condensed chromatin from normal tissues loses its specific properties readily on reprecipitation, DNP from tumor tissues possesses stable inherent characteristics which are evidently attributable to the low probability of dissociation (up to $\mu = 1$) of the component endowing it with these specific properties.

It can be postulated on the basis of these results that during malignant transformation there is irreversible heterochromatization of some of the diffuse chromatin (in the normal cell this process is evidently reversible). The increase in the fraction of heterochromatin can be regarded as a reflection of the decrease in the quantity of genetic information translated in the tumor cell. At the same time, this hypothesis is not contradicted by results showing the local unblocking of certain areas of the genome during malignant transformation which do not function in the homologous tissue but which are active in other specialized tissues.

LITERATURE CITED

1. L. G. Kulikova, in: Proceedings of a Conference of Junior Scientists of the Institute of Experimental Biology, Academy of Medical Sciences of the USSR [in Russian], Moscow (1961), p. 9.
2. E. M. Leikina, L. K. Romanova, D. M. Spitkovskii, et al., in: Proceedings of the 6th Conference to Review the State of Recent Research into Processes of Regeneration and Cell Division [in Russian], Moscow (1971), p. 94.
3. A. S. Spirin, Biokhimiya, 23, 656 (1958).
4. D. M. Spitkovskii, Biofizika, 3, 396 (1958).
5. D. M. Spitkovskii, Biokhimiya, 20, 566 (1955).
6. D. M. Spitkovskii, The Radiosensitivity of Deoxyribonucleoproteins of Somatic Cells as a Function of Their Structural Organization, Doctoral Dissertation, Moscow (1970).
7. D. M. Spitkovskii, V. T. Andrianov, and A. N. Pisarevskii, Radiation Biophysics of Nucleoprotein [in Russian], Moscow (1969), p. 110.
8. D. M. Spitkovskii and L. A. Luchkina, Biofizika, 11, 747 (1966).
9. V. C. Allfrey, A. C. Mirsky, and S. Osava, J. Gen. Physiol., 40, 451 (1957).
10. H. Bielka, A. Henske, and I. Schneiders, Acta Biol. Med. Germ., 13, 161 (1964).
11. H. Bielka and J. Junghahn, Exp. Cell Res., 54, 2 (1969).
12. H. Busch and W. Starbuck, Ann. Rev. Biochem., 33, 519 (1964).
13. H. Grunicke, R. van Potter, and H. P. Morris, Cancer Res., 30, 776 (1970).
14. E. Harbers and M. Vogt, in: Proceedings of the International Symposium on the Cell Nucleus. Metabolism and Radiosensitivity, London (1966), pp. 165, 173.
15. S. Kit, Arch. Biochem., 87, 318 (1960).
16. S. Kit, Arch. Biochem., 87, 330 (1960).
17. O. H. Lowry, N. J. Rosebrough, A. Farr, et al., J. Biol. Chem., 193, 265 (1951).
18. J. McCarthy and B. H. Hoyer, Proc. Nat. Acad. Sci. (Washington), 52, 915 (1964).
19. A. E. Mirsky and A. U. Pollister, J. Gen. Physiol., 30, 17 (1946).
20. M. A. O'Neal and V. C. Ward, Proceedings of the 5th International Biochemical Congress [in Russian], Vol. 2, Moscow (1963), p. 27.
21. J. Shack, R. J. Jenkins, and J. M. Thompsett, J. Nat. Cancer Inst., 13, 1435 (1953).
22. J. Shack and J. M. Thompsett, J. Nat. Cancer Inst., 13, 1425 (1953).