STRUCTURAL CHANGES IN NUCLEAR CHROMATIN FRACTIONS OF RAT LIVER CELLS

D. M. Spitkovskii and E. M. Leikina

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A comparison was made of the chemical composition and thermomechanical properties of the chromatin and its fractions (condensed and diffuse) obtained from rat liver cell nuclei. The diffuse chromatin fraction gave the highest protein/DNA ratio and the highest RNA content. The fractions differed considerably in their degree of spontaneous contraction after isolation: diffuse chromatin gave the highest value. Flowability within the range 60-80°C was maximal for the diffuse and minimal for the condensed chromatin. The amplitude of the transition for diffuse chromatin was 55% and for condensed 30%. These properties point to a higher degree of intermolecular interaction in the condensed chromatin. A formula is given for calculating the quantity of condense and diffuse chromatin in whole chromatin. A hypothesis is put forward for the possible structural character of repression.

The chromatin of interphase nuclei in the tissues of higher animals can exist in two structural states [7, 8]: condensed and diffuse, with different functional activity.

Some information had been obtained [1, 9] on the composition and properties of these fractions isolated by Frenster's method [7], that which is most widely used. However, the physicochemical properties of these fractions, reflecting their possible structural state in the cell, have received virtually no investigation, for at physiological ionic strengths the chromatin is an insoluble substance (which accounts for the phase demarcation of the chromosomes), so that it is difficult to investigate by ordinary physicochemical methods. Meanwhile the analysis of the structural parameters of chromatin is of the greatest interest for it would shed some light on the relationship between structural organization and functional activity of this substrate.

One of the purposes of this investigation was to make an experimental analysis of the structural parameters of the supramolecular systems of condensed and diffuse chromatin in a medium of physiological ionic strength. It was hoped that such an analysis would help to elucidate some of the principles governing the condensation processes which play an important role in the structure of chromosomes and in the mechanism of regulation of genetic activity.

EXPERIMENTAL METHOD

The test object consisted of the liver of noninbred male rats weighing 160-180 g. Nuclei from the liver were isolated and purified by the method of Chauveau et al. [6] and the purity of the nuclei was verified cytologically. The chromatin was fractionated by the method of Frenster et al. [7]. The protein content in the chromatin and its fractions was determined by the method of Lowry et al. [10]. DNA and RNA were separated by the method of Schmidt and Thannhauser [11], and their concentration was determined spectrophotometrically after alkaline hydrolysis for RNA and acid hydrolysis for DNA [3]. The structure of DNA in the chromatin and the properties of chromatin as the result of interaction between DNA and its other components were investigated by a thermomechanical method [4], yielding information on the "helix coil" transformation of the DNA molecules in the chromatin and its fractions and on the character of interaction

Laboratory of Molecular Biology, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.)

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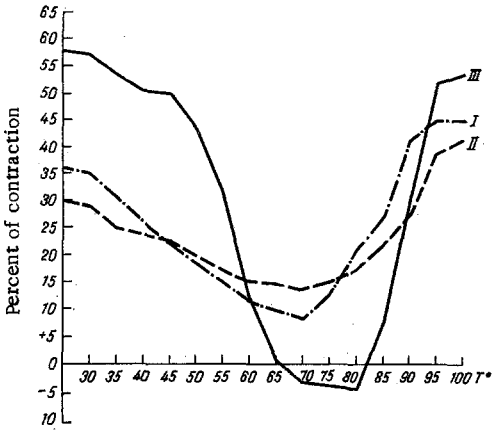

Fig. 1

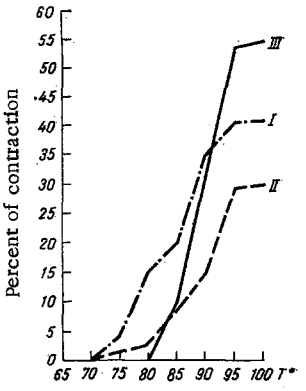

Fig. 2

Fig. 1. Relative elasticity of fibers of chromatin and its fractions as a function of temperature: i) whole chromatin; ii) condensed chromatin; iii) diffuse chromatin (medium 0.14 M NaCl, pH 6.8-7.0).

Fig. 2. Curves of structural changes in fibers of chromatin and its fractions in the region of the "helix-coil" transformation of DNA. Legend as in Fig. 1.

between the molecules in the supramolecular structure in a medium of physiological ionic strength. The essentials of the method, the techniques used to obtain supramolecular orientated structures, and the calculation of the structural parameters of the chromatin systems were similar to those described previously for DNP-systems [5]. Each point on the graft (Figs. 1 and 2) reflects mean values obtained for three fibers. The error does not exceed ±1.5%.

EXPERIMENTAL RESULTS

The results of the study of the chemical composition of rat liver nuclear chromatin and its fractions are given in Table 1.

These results show that the highest value of the protein/DNA ratio and highest RNA content were found for the fraction of diffuse chromatin, in agreement with results reported elsewhere [1, 7, 9].

The relative elasticity of the fibers of chromatin and its fractions is shown in Fig. 1 as a function of temperature determined by a thermomechanical method. It follows from the grafts that the fractions differed considerably in their degree of spontaneous contraction of the fibers immediately after isolation. Diffuse chromatin showed the highest value. The fractions also differed in their flowability [4] in the interval 60-80°C: it was minimal for condensed and maximal for diffuse chromatin. These properties point unequivocally to stronger molecular interaction in the condensed chromatin. Significant differences also were observed in the region of the "helix-coil" transition of the DNA in the chromatin fractions. It will be clear from Fig. 1 (the right, ascending part of the curve) and Fig. 2 that the corresponding transformation for condensed chromatin begins at lower temperatures than for diffuse. The amplitude of the transformation reached 55% for diffuse and only 30% for condensed chromatin.

The results obtained by the thermomechanical method thus led to the following conclusion regarding the structure of condensed chromatin: a smaller degree of initial contraction of the fibers, lower flowability, a smaller amplitude of "helix-coil" transformation, a less cooperative transformation and, finally, its earlier onset – all these indicate that the DNP molecules in the chromatin possess certain distinguishing features which contribute to their denser packing in the system.

At the same time it must be emphasized once again that the protein content (relative to DNA) in condensed chromatin is less than in diffuse. The condensation effect is thus not due to an excess of protein, as was hitherto considered, but probably the highest content of an as yet unidentified component. It may be that the different qualitative composition of the protein plays a role in this phenomenon. As well as indicating a role of the different protein composition in the diffuse chromatin, these results also support the

TABLE 1. Chemical Composition and Some Physicochemical Properties of Chromatin and Its Fraction

Chromatin	Protein/ DNA	RNA (in % of DNA)	$E_{260}^{(p)}$	dl/g η
Whole	2,26	10,2	6370	53
Condensed	2,32	5,8	6516	32
Diffuse	2,67	17,1	6854	30

hypothesis that repression, to a large extent at least, is not due to direct chemical blocking of the template but is structural (stereochemical) in character. That repression may take place in this way was hinted at previously after analysis of the action of actinomycin on the DNP-system [2]. In the composition of DNP it can be assumed that areas capable of active synthesis of messenger RNA are constantly present, although the level of their accessibility is controlled by condensation processes.

Finally, the results obtained by the thermomechanical method provide a means of accessing the relative proportion of condensed and diffuse components in the composition of chromatin. Assuming that the relaxation parameters of whole chromatin are additive with respect to those for its condensed and diffuse components, we can write: $a_1K+a_2D=a_3$, and $K+D=1$, where a_1, a_2, and a_3 are the amplitudes of initial contraction of the condensed, diffuse, and whole chromatins respectively; K and D are the fractions of condensed and diffuse chromatin. The values of a_1, a_2, and a_3 can easily be determined from Fig. 1, and by means of the equation given above this readily gives the values K = 80% and D = 20%. These values agree closely with those reported in the literature [7].

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