COMPUTER ANALYSIS OF DATA ON CHROMATIN FRAGMENTATION BY NUCLEASES

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Interpretation of the results of chromatin fragmentation by nucleases is usually based on analysis of the distribution of the sizes and numbers of DNA and DNP fragments on electrophoresis in agarose and polyacrylamide gels or during centrifugation in density gradients. Better fractionation is achieved by electrophoresis, although in this case quantitative determination of DNA in the gels may give rise to some difficulty. This is because of methods of DNA detection in gels [6], the heterogeneity of chromatin endonucleolysis products [4, 7], and the considerable overlapping of the peaks on the densitograms [2].

This paper describes the use of computer analysis of densitograms aimed at picking out individual peaks from the composite graph of RNA distribution in the gels, and the dynamics of accumulation of nucleosomes and their oligomers in the course of chromatin fragmentation by endogenous nuclease was compared with a stochastic model of this process.

EXPERIMENTAL METHOD

Methods of isolation of the nuclei, fragmentation of chromatin DNA by nucleases, and isolation and electrophoresis of the DNA were described previously [1]. The DNA content and conditions of photography of the gels were chosen in preliminary experiments so as to ensure that transmission of the DNA content was proportional to the amplitude of the corresponding wave of the densitogram. Scanning of negatives or of the gels themselves (under fluorescence conditions) was carried out on a Chromoscan-200—Scan-201 system (Joyce-Loebl, England). Processing of individual blocks of programs and ongoing processing of the results were carried out on HP-67 (Hewlett-Packard, USA) and TI-59 (Texas Instruments, USA) minicomputers. Complete analysis of the densitograms and simulation of chromatin fragmentation were done on the BESM-6 and ES-1022 computers.

EXPERIMENTAL RESULTS

A typical densitogram, showing the distribution of DNA fragments isolated from chromatin, degraded by endogenous nuclease from rat liver nuclei, in 2% agarose gel is shown in Fig. 1.

Clearly only the mononucleosomal fragments of DNA are sufficiently clearly separated; peaks corresponding to their multiplets overlap, and the higher their serial number, the more they overlap. Graphic analysis of curves of this type is difficult, or can be applied only to well-separated mono- and dinucleosomes. More objective information can be obtained by analyzing the composite curves into components. The simplest approach is to present the resultant curve in the form of the sum of a series of gaussoids, each of which describes the distribution of a homogeneous preparation of mononucleosomes or a definite multiplet of them in the gel. Adequacy of description can be assessed by the method of least squares, assuming that if the calculated resultant and the experimental curve coincide the positions and areas of the calculated gaussoids will correspond precisely to the dsitribution of individual types of DNA fragments in the densitogram under analysis.

The task is thus reduced to its simplest form of seeking a function $\varphi_T(x)$ which minimizes the expression:

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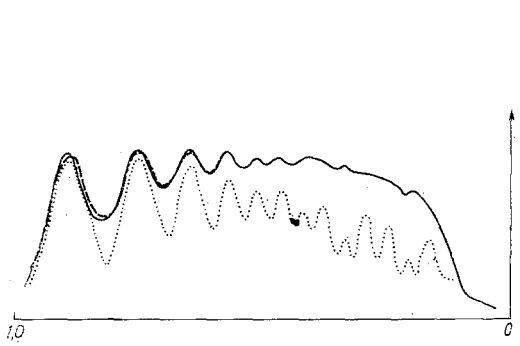

Fig. 1

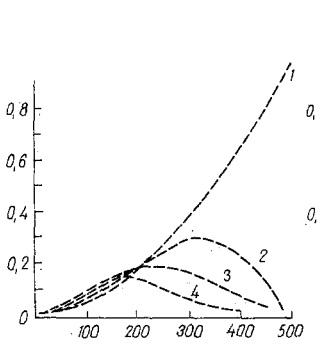

Fig. 2

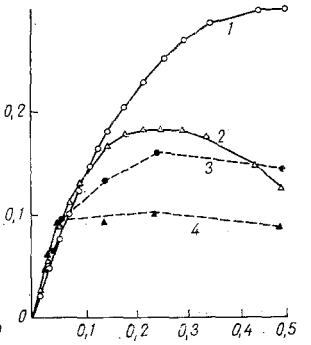

Fig. 3

Fig. 1. Distribution in 2% agarose gel of DNA fragments isolated from chromatin degraded by endogenous nuclease. Continuous line — experimental graph, broken line — resultant of selected gaussoids, dotted line — envelope of gaussoids. Abscissa, mobility (in relative units); ordinate, optical density (in relative units).

Fig. 2. Fragmentation of 500-nucleosomal segments of chromatin according to computer simulation data. 1, 2, 3, 4) Mono-, di-, tri-, and tetranucleosomes. Abscissa, number of cleavages of internucleosomal DNA segments; ordinate, content of mononucleosomes and their oligomers in total hydrolysate of chromatin.

Fig. 3. Dependence of formation of di- and tri-nucleosomes on content of mononucleosomes in total hydrolysates of chromatin. 1, 2) Di- and trinucleosomes respectively based on stimulation data, 3, 4) di- and trinucleosomes based on experimental data. Abscissa, content of mononucleosomes in chromatin hydrolysate; ordinate, content of di- and trinucleosomes.

$$R = \int_{x=0}^{x=l} [\varphi_T(x) - \varphi_э(x)]^2 \, dx,$$

where R is a measure of the deviation of $\varphi_T(x)$ from $\varphi_e(x)$, $\varphi_e(x)$ is the experimental curve represented by the densitogram, and $\varphi_T(x)$ is the resultant, i.e., the sum of the calculated gaussoids:

$$\varphi_T(x) = \sum_{i=1}^{N} P_i \cdot \frac{1}{\sigma_i \sqrt{2\pi}} \cdot e^{-\frac{(x-m_i)^2}{2\sigma_i^2}},$$

where N is the number of gaussoids, P_i the area beneath the graph of the i-th gaussoid, m_i the position of the maximum of the i-th gaussoid, and δ_i its effective width.

The algorithm used was based on the fact that the required values of P_i, m_i, σ_i ($i = 1, \ldots, N$), minimizing R, convert the corresponding derivatives dR/dP_i, dR/dm_i, $dR/d\sigma_i$ to zero. The solution was done by iteration methods on the computer; for the fastest search for the corresponding values each subsequent approximation was obtained from the preceding one by a shift in the direction opposite to the gradient R (i.e., the vector value showing the direction of the most rapid increase in the function R). The program was composed in FORTRAN language. The algorithm used ensures good agreement between the experimental and theoretical curves over 10-13 iterations. It will be clear from Fig. 1 that with an increase in the serial number of oligomer differences between its true distribution and area (estimated from the corresponding gaussoids) and apparent distribution corresponding to the peak on the densitogram increased. Meanwhile the algorithm used does not discriminate regions of the densitograms containing discrete material of oligomers and nondiscrete high-molecular-weight zones, and in consequence of this only gaussoids corresponding to mononucleosomes and their oligomers up to hexanucleosomes inclusive were used in the

calculation. By using this type of method to analyze the densitograms it was possible to determine the dynamics of accumulation of mononucleosomes and their multiplets during cleavage of chromatin by endogenous nuclease. It might be supposed that this dynamics ought to reflect the character of distribution of nucleosomes in DNP-fibrils and, consequently, the distribution of regions accessible for nuclease.

To evaluate the experiments, endonucleolysis of chromatin was simulated by a computer model based on the hypothesis of uniform distribution of nucleosomes and the random character of fragmentation of DNP-fibrils. The assumptions lying at the basis of the simplest stochastic model of endonucleolsis of chromatin, in more detail, are as follows: 1) the DNP-fibril is an open chain of nucleosomes without self-intersections; 2) all nucleosomes and connections between them are identical. Two oligomers of equal length, removed from different ends of a DNP-fibril are regarded as equivalent; 3) cleavages take place in succession, although the time interval between them may be less than any preassigned number ε; 4) the system is closed; 5) the end product of cleavage is mononucleosomes.

If these assumptions are accepted it can be shown that the state of the system after each double-stranded cleavage of DNA can be described as:

$$M_i(n+1) = M_i(n) + \frac{2}{m(k-1)-n} \sum_{l=i+1}^{l=k} M_l(n) - \frac{(i-1)M_i(n)}{m(k-1)-n}$$

for i = 1, 2, ..., k − 1,

$$M_k(n+1) = M_k(n) - \frac{(k-1)M_k(n)}{m(k-1)-n}$$

for i = k,

where K is the number of nucleosomes in the original m-fragments of chromatin, n is the number of double-stranded breaks in DNA, M_i, M_1, M_k the mathematical expectancy of the number of chromatin fragments of the corresponding size. The results of the computer calculations are shown in Fig. 2 as a graph of content of monotetranucleosomes in chromatin hydrolysates as a function of the number of double-stranded breaks in DNA.

The data obtained by the model are compared in Fig. 3 with the experimental results; the content of mononucleosomes in total chromatin hydrolysates was taken as the unit of measurement of the degree of fragmentation of chromatin during this comparison. It will be clear from Fig. 3 that in the experiment, for a given number of mononucleosomes, the yield of di- and trinucleosomes was less than that predicted by the model. This points to a certain "narrowing of the bounds" of the action of endogenous nuclease, leading to an increase (compared with the stochastic variant described by the model) in the probability of formation of mononucleosomes by a single double-stranded cleavage of DNA. In this connection it must be noted that a change in the original size of the degraded chromatin fragments in the model calculation does not lead to any change in the relations between the number of mononucleosomes and their multiplets (according to the results of calculations for 500-, 50-, and 20-nucleosomal segments). Consequently, the differences observed ought to be connected with a definite specific character and, possibly, heterogeneity of structure of the chromatin [3-5, 7, 8].

We consider that the further development of this approach, combining conputer analysis of experimental data on degradation of chromatin by nucleases with the construction of mathematical models of this process, can provide certain characteristics and classification parameters for the structural components of chromatin.

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