MACROMOLECULAR ORGANIZATION OF DNA IN REGENERATING RAT LIVER AFTER PARTIAL HEPATECTOMY

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The primary structure of DNA was studied during the period of its intensive synthesis, using DNA from the regenerating rat liver as example. In the pre- and postsynthetic period, virtually no changes occurred in the polynucleotide strands of DNA. In DNA preparations isolated during passage through the S period, the density of single breaks (probability of their formation) is much higher than normally.

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According to data in the literature, the biological function of DNA is associated with its definitive physicochemical state. It has been shown, for instance, that DNA isolated from various organisms (viruses, bacteria, animals, and plants) is completely or partially denatured in the period of replication [14, 15, 17]. Some polymerases are also known to utilize only denatured forms of DNA as primer for replication in vitro [7]. The main difficulty when studying the physicochemical state of DNA during these vitally important functions is the need for large quantities of homogeneous material. This condition can be satisfied only in the case of cultures synchronized with respect to their cycle of division.

A convenient object for investigation of the state of DNA in various stages of the mitotic cycle is the regenerating liver. Partial hepatectomy is a stimulus to cell division—after the operation, intensive preparation for mitosis begins in the cells of the residual part of the liver, and to some extent they pass through all periods of the mitotic cycle synchronously. Interesting data regarding the state of the secondary structure of DNA in different stages of the mitotic cycle, with DNA from regenerating rat liver as the example, were obtained by Salganic and co-workers [2, 16]. They showed that DNA, isolated from regenerating rat liver 24 h after hepatectomy, i.e., the period of most intensive replication, is partially (by 10-15%) denatured. This injury to the hydrogen bonds is not revealed by the profile of the melting curves, but can be detected only by interaction between the denatured groups and formaldehyde or SME-carbodiamide, or by their fractionation with chloroform.

In the present investigation possible changes in continuity of the primary structure of DNA (the appearance of single breaks in its polynucleotide chains) in a period of intensive DNA synthesis were studied, using as example DNA from regenerating rat liver after partial hepatectomy.

Such an investigation is interesting from two viewpoints. First, it is known that zones of melting of hydrogen bonds can form around single breaks [5]. Quantitative determination of these defects can thus help to elucidate the mechanisms leading to the appearance of denatured forms of DNA in vivo. Second, in recently published papers [12, 13], data are given indicating that single breaks appear during replication of phage and bacterial DNA. It was therefore important, in principle, to demonstrate that common changes can take place in DNA in objects possessing different levels of biological organization.

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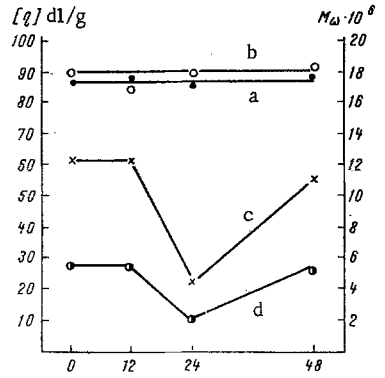

Fig. 1. Changes in characteristic viscosity and, consequently, in molecular weight of DNA from regenerating liver isolated at various times after partial hepatectomy. Abscissa, time after operation (in h), ordinate (left) – characteristic viscosity, (right) – molecular weight; a) characteristic viscosity of NDNA; b) molecular weight of NDNA; c) characteristic viscosity of SDNA; d) molecular weight of SDNA.

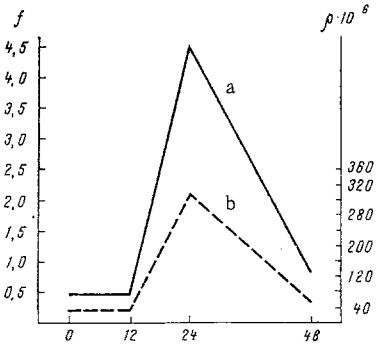

Fig. 2. Accumulation of single breaks in DNA molecule during period of intensive DNA synthesis. Abscissa, time after operation (in h), ordinate (left) number of breaks in DNA molecule (f), (right) density of breaks in DNA molecule (ρ); a) number of breaks; b) density of breaks.

TABLE 1. Accumulation of Breaks in DNA Macromolecule at Various Times after Operation

Time after partial hepatectomy (in h)	$f_t - f_0$	Significance of difference	$\rho_t - \rho_0$	Significance of difference
12	0	No difference	0	No difference
24	4,0	Significant (t > 3)	$300 \cdot 10^{-6}$	Significant (t > 3)
48	0,3	Not significant (t < 3)	$21 \cdot 10^{-6}$	Not significant (t < 3)

EXPERIMENTAL METHOD

DNA was isolated from the liver of male noninbred albino rats weighing 130-180 g by Georgiev's phenol method [1], which is regarded as blocking enzyme systems including those which could lead to destruction of the DNA macromolecule. Partial hepatectomy was carried out by the method of Higgins and Anderson [10]. The following times were chosen for isolation of DNA after partial hepatectomy; 12, 22-24, and 48 h. The time of 22-24 h after operation was chosen on the basis of results obtained by Dashkevich and coworkers [2], according to whom changes in the secondary DNA structure reached a maximum at this period. The DNA concentration was determined by Spirin's method [4], protein by Lowry's method [11], and RNA by the Schmidt–Thannhauser–Schneider method. The molecular weight of the DNA preparations was estimated viscosimetrically in a low-gradient viscosimeter of the Ostwald type with flow gradients of 60, 40, and 20 sec^{-1}. To calculate the molecular weight of native DNA (NDNA) the formula suggested by Crother and Zimm [8] was used. Separation of the DNA strands was carried out in phosphate buffer, pH 7.0, by 0.01 M Na^+ [9] at a temperature of 92-94°, followed by rapid cooling. When calculating the molecular weight of single-stranded DNA (SDNA) on the basis of the characteristic viscosity ($[\eta] = KM^\alpha$) it was assumed that $\alpha = 0.94$ and $K = 26 \cdot 10^{-6}$. These values of α and K were suggested by Eigner and Doty [9] for single-stranded DNA preparations with a G-C content of 42%; the content of G-C pairs in rat liver is about 42-45% of the total content of bases [18]. The melting temperature of DNA was measured on the SF-4 spectrophotometer in thermostatically controlled quartz cells.

All results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Comparison of the characteristic viscosities of DNA preparations isolated at various times of regeneration, and the molecular weights calculated from them, revealed no statistically significant differences from normal (Fig. 1). The preparations of double-stranded DNA obtained before and after partial hepatectomy were characterized by equal values of melting temperature and identical shapes of the melting curves ($T_m = 82 \pm 2°$ at an ionic strength of 0.2), and also by an identical percentage RNA content (4.5 ± 2%). This does not mean, however, that in the period of DNA replication no changes take place in its polynucleotide strands. To investigate the state of the primary structure of DNA at a period of intensive DNA synthesis, and also in the pre- and postsynthetic period, DNA strands were separated and certain molecular parameters of

single-stranded segments were determined. After heat treatment of NDNA, in every case typical single polynucleotide chains (PG ≥ 15 and TPV ≥ 3) [5] were obtained in every case, so that when their characteristic viscosity was compared, this gave an estimate of the degree of continuity of the primary DNA structures.

Knowing the characteristic viscosity of NDNA and of the corresponding SDNAS, the number of single breaks in the DNA chains formed by isolation of NDNA through heat treatment during separation of the DNA strands, or for other reasons, can be calculated. The number of single breaks leading to degradation of the polymer is determined from the formula [6]:

$$f = \frac{M_n^{theor}}{M_n^{exp}} - 1,$$

where f represents the number of breaks, M_n^{theor} the mean theoretical molecular weight of SDNA, and M_n^{exp} the mean molecular weight of SDNA obtained experimentally. Provided that

$$\alpha = 0.94 \qquad M_n = \frac{M_w}{1.97}$$

or

$$f = \frac{\frac{M_W^{theor}}{1.97}}{\frac{M_W^{exp}}{1.97}} - 1,$$

then

$$f = \frac{M_W^{theor}}{M_W^{exp}} - 1,$$

where M_W represents the weighted mean molecular weight.

From the value of f the number of breaks can be determined not in the whole NDNA molecules, but only in that part of the polynucleotide chain which possesses a definite characteristic viscosity and a definite weighted mean molecular weight, calculated from the formula:

$$[\eta] = 26 \cdot 10^{-6} M^{0.94}.$$

To obtain more comparable results, the density of fragmentation of this bond under the experimental conditions used was therefore calculated. The value of ρ (the density of the breaks) was determined by formula [4]:

$$\rho = \frac{2f \cdot w}{M_w^0},$$

where w is the mean molecular weight of the nucleotide or 340.

Values of single breaks and densities of single breaks in polynucleotide strands of DNA isolated at different times after partial hepatectomy (12, 24, and 48 h) are given in Fig. 2. It will be clear from Fig. 2 and Table 1 that in DNA isolated 24 h after hepatectomy, i.e., at a time of intensive DNA synthesis, the density of single breaks was significantly higher than in DNA isolated from the intact liver and also 12 and 48 h after hepatectomy.

Knowing the probability of appearance of single breaks, the number of them which can be formed in a molecule with a definite molecular weight can be calculated. For example, in a DNA molecule with molecular weight of about $18 \cdot 10^6$ about 4.5 single breaks may arise in the S period, while in the pre- and post-synthetic period the mean number of breaks per molecule is 0.5.

The mechanism of formation of single breaks and their subsequent repair is not yet known. Tissue deoxyribonucleases possibly play an important role in the formation of these breaks. In later periods after the operation, repair evidently takes place in the DNA molecules, possibly through cross-linking enzymes.

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