INVESTIGATION OF LOCAL RADIATION INJURIES IN DNA BY THE METHOD OF THERMAL SEPARATION OF SPIRALS

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Research during recent years has shown that irradiation of DNA with small and moderate doses of ionizing radiation is not followed by any fall in molecular weight detectable by the light-dispersion method [6, 12]. This is not to exclude the possibility that local disturbances may occur within the molecular structure.

Among such possible injuries may be included single breaks in the phosphoric ester chains and the formation of intramolecular cross-linkages. It is evident that either of these may have definite biological effects, leading in particular to rupture of the coding chain and to loss of the power of reduplication.

Alexander [4] has shown that if DNA is irradiated by a stream of electrons in a dose of the order of hundreds of thousands of roentgens, single ruptures may be produced in the polynucleotide chains. An investigation by Marmur [9] has demonstrated the formation of intramolecular thermostable cross-linkages as a result of ultraviolet irradiation of DNA.

One of the most effective approaches to the investigation of internal injuries in the DNA macromolecule is the study of single-strand DNA obtained from irradiated preparations. For this purpose it is necessary to determine the molecular weight, the number of chains, and the degree of flexibility of both the original DNA and the products obtained from it as a result of separation of the chains.

The number of chains in the DNA macromolecule may be determined by studying the kinetics of its degradation by ionizing radiation. If the change in viscosity is taken as the criterion of degradation, the degradation of linear polymers may be described (if it is assumed that the breaking of the chain obeys the law of chance) by the equation

$$\log [\eta] = -\alpha \log (R_0 + R) + \text{const},$$

where $[\eta]$ is the characteristic viscosity of the irradiated preparation; α the index of degree in Staudenger's equation, R_0 the dose required in order to break a semi-endless chain to the initial distribution by molecular weights, and R the dose of irradiation [5]. If the molecule consists of two chains, like, for example, untreated double-strand DNA, the above equation will assume the following form:

$$\log [\eta] = -2\alpha \log (R_0 + R) + \text{const},$$

where the multiplier 2 indicates the number of strands in the DNA [7]. If this equation is generalized for n chains, the multiplier 2 is replaced by n, and then if $R \gg R_0$, the tangent of the angle of deviation of the linear part of the graph of the relationship between $\log [\eta]$ and $\log (R)$, divided by $-\alpha$, will be equal to the number of strands in the DNA molecule.

According to Doty [8], α for single-strand DNA in a solution of 0.01 M Na^+ is 0.92; α for double-spiral DNA in a solution of 0.2 M Na^+ is 1 [3, 7].

Single-strand DNA must differ from double-spiral DNA not only by its particular type of kinetics of degradation, but also by its physico-chemical properties. Their structural differences are evidently manifested as differences in flexibility. In contrast to double-strand DNA, single-strand must change its configuration easily in

accordance with the external environmental conditions (ionic strength of the solution, temperature, etc.). A characteristic property of single-strand DNA is the increase in its viscosity when the temperature rises, as a result of the opening out of its molecule. The same property is possessed, for example, by RNA which is a single-strand structure [2]. No change takes place in the viscosity of double-spiral DNA in these conditions.

Comparison of the viscosity of single-strand DNA obtained from irradiated and nonirradiated DNA may give information on the presence of breaks in the DNA chain caused by irradiation. The viscosity of single-strand DNA obtained from DNA in whose chain there are several breaks must be greatly reduced by comparison with the viscosity of the single-strand DNA isolated from nonirradiated double-spiral DNA.

We therefore started our investigation from the standpoint that by determining the number of strands from the kinetics of degradation, and by studying the viscosity and other properties of the single-strand structures obtained from irradiated DNA preparations, we could evaluate the local injuries to the DNA macromolecule caused by radiation.

EXPERIMENTAL METHOD

Double-spiral DNA was isolated from a calf's thymus; preliminary isolation of DNA was by Mirsky and Pollister's method [10] and subsequent deproteinization with phenol by Georgiev's method [1]. The preparations had the following characteristics: N/P $\cong$ 1.64–1.68; E(P) = 260 mμ = 6500-6700; molecular weight = $7 \cdot 10^6$-$8.5 \cdot 10^6$ [3].

Roentgen-ray irradiation was given by means of a type RUM-60 apparatus (filter 0.5 mm Al, tube voltage 60 kV, dose rate 5000 r/min). Ultraviolet irradiation was given by means of an apparatus assembled on a BUV-15 lamp according to a scheme suggested by Alexander [11]. The duration of irradiation was 5 min and the power $4.7 \cdot 10^4$ erg/min $\cdot$ mm^2.

We obtained single-strand DNA from irradiated and nonirradiated preparations by Doty's method [8] at 88°. The viscosity was determined by means of a low-gradient viscosimeter of the Ostwald type. The coefficient of compression of the single-strand DNA was determined during transfer from a solvent with 0.01 M Na^+ into a solvent with 0.2 M Na^+, i.e., we determined the ratio $\frac{[\eta]_{0.01\ M\ Na^+}}{[\eta]_{0.2\ M\ Na^+}}$. The viscosity of single-strand DNA in 0.2 M Na^+ was measured at 25° and 70°, and the ratio $\frac{[\eta] \text{ in } 0.2\ M\ Na^+\ (70°)}{[\eta] \text{ in } 0.2\ M\ Na^+\ (25°)}$ was determined.

EXPERIMENTAL RESULTS

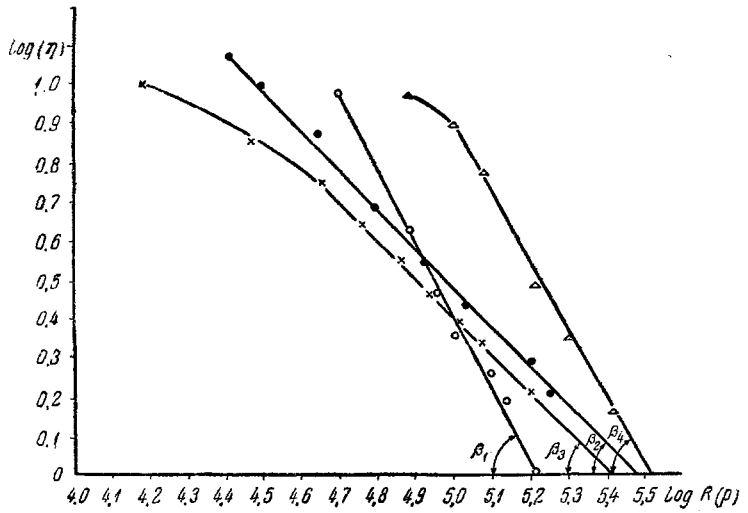
The results of determinations of the kinetics of radiation degradation are given in the figure and show that the number of strands in double-spiral DNA was close to 2; this reflected its double-spiral structure. The number of

Relationship between $[\eta]$ and Na^+ Concentration and Temperature of Solution

Preparation	No. of preparation	$[\eta]$ of single-strand DNA obtained from nonirradiated DNA / $[\eta]$ of single-strand DNA obtained from irradiated DNA	$[\eta]$ 0.01 M Na^+ / $[\eta]$ 0.2 M Na^+	$[\eta]$ in 0.2 M Na^+ (70°) / $[\eta]$ in 0.2 M Na^+ (25°)
Single-strand DNA obtained from nonirradiated DNA	1	–	32	3.7
	2	–	27	3.0
Single-strand DNA obtained from DNA irradiated in a dose of 7500 r	3	4	22	3.7
	4	3.4	21	3.1
Single-strand DNA obtained from DNA irradiated with ultraviolet light (5 min)	5	3.8	5	1.0
	6	3.7	5	1.0

strands in the structures obtained from preparations irradiated with roentgen rays and from nonirradiated preparations by heating as in Doty's method was 1.08. Consequently, the structures in fact were basically single-stranded.

It may be concluded from these findings that thermostable cross-linkages between DNA chains do not develop during roentgen-ray irradiation. In contrast to roentgen-ray irradiation, ultraviolet irradiation prevents the separation of the spirals, evidently as a result of the formation of thermostable cross-linkages between the DNA chains. The tangent of the angle of inclination of the straight-like part of the curve was actually not -1, but -1.6, suggesting that the preparation contained a considerable amount of broken spirals. Cross-linkages not only prevent separation of the



Degradation of preparations by roentgen rays. O—Double-spiral DNA ($n = \frac{tg\beta_1}{-\alpha} = \frac{1.86}{1} = 1.86$); ●—single-strand DNA, isolated from a nonirradiated preparation ($n = \frac{tg\beta_2}{-\alpha_2} = \frac{1.0}{0.92} = 1.08$); +—single-strand DNA isolated from double-spiral DNA irradiated with roentgen rays (7500 r) ($n = \frac{tg\beta_3}{-\alpha_3} = \frac{0.99}{0.92} = 1.06$); ▲—structure isolated from double-spiral DNA irradiated with ultraviolet light ($tg\beta_4 = -1.6$). Kinetics of degradation of preparations of single-strand DNA isolated from nonirradiated DNA and from DNA irradiated with roentgen and ultraviolet rays in the presence of 0.001 M EDTA.

spirals, but also strengthen the structure a little, for the region of roentgen-ray degradation of the preparations irradiated with ultraviolet light was shifted towards the large doses. Complete correlation was observed between the degradation kinetics and the results of the study of the viscosity at different electrolyte concentrations and temperatures (see table).

We thus see that in the case of single-strand DNA obtained from nonirradiated DNA or DNA irradiated with roentgen rays, an increase in the concentration of sodium ions from 0.01 M to 0.2 M led to the rolling of the molecule into a ball. This process was accompanied by a 20-30-fold decrease in the viscosity. Flexibility of this sort is characteristic of certain linear nonelectrolytes. Double-spiral DNA possesses considerable rigidity, and in these conditions its viscosity changes by a factor of only 1.2-1.5 [8]. In a 0.2 M Na^+ solution, a rise of temperature from 25 to 70° leads to an increase in the viscosity of the single-strand DNA solution by more than three times. Under the same circumstances the viscosity of untreated DNA remains constant.

These results emphasize the single-strand structure of these polymers. Nevertheless, the viscosity of the single-strand DNA obtained from DNA irradiated with roentgen rays is lower than that of DNA obtained from a nonirradiated preparation by 67-75%. Irradiation apparently causes breaks along the chains of the DNA molecule which reveal themselves by a separation of the spirals. When a dose of 7500 r is given, there are comparatively few of these breaks (irradiation in the presence of 0.001 M EDTA).

The structure obtained from DNA irradiated with ultraviolet light possesses much less flexibility in the presence of an increased concentration of Na^+ ions or a raised temperature, and resembles the DNA denatured in 0.2 M NaCl, consisting of two cross-linked strands [13].

SUMMARY

By the method of thermal separation of strands a study was made of the local injuries in the DNA macromolecule caused by x-ray and ultraviolet irradiation. By analysis of the degradation kinetics and flexibility of the initial and final products it was shown that A-irradiation causes no cross linking preventing the separation of DNA strands; at the same time irradiation leads to the appearance of solitar breaks in polynucleotide chains. In ultraviolet irradiation thermostable cross-linking is formed, preventing the separation of the two-stranded structure.

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