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Studies on Homopolymer Synthesis with Irradiated DNA in the RNA Polymerase System

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Studies on homopolymer synthesis with irradiated DNA in the RNA polymerase system

Preparations of RNA polymerase (RNA nucleotidyltransferase, EC 2.7.7.6), isolated from *Escherichia coli* according to the method of CHAMBERLIN AND BERG[1], catalyse, apart from the synthesis of RNA, the synthesis of poly (A) and poly (U) in the presence of primer DNA, ATP and UTP, respectively. In connection with earlier investigations into the priming ability of irradiated DNA[2–5], its activity was studied with respect to homopolymer synthesis. Calf-thymus DNA was irradiated either with ^{60}Co γ-rays (30 000 R, 500 μg/ml, 0.01 M NaCl) or with ultraviolet light ($4.42 \cdot 10^8$ erg/ml, measured with the aid of uranyl acetate[6]). These DNA samples will be abbreviated to "γ-DNA" or "UV-DNA", respectively. Further experimental details have been described previously[3,4].

The effect of various doses of γ-irradiation on native DNA is shown in Table I. Small doses lead to an increase of poly A synthesis, high doses to a decrease. This is in

TABLE I

EFFECT OF γ-IRRADIATION ON THE PRIMING ABILITY OF DNA FOR POLY (A) SYNTHESIS

The incubation mixture (0.25 ml) contained in μmoles: Tris buffer (pH 7.9), 10; $MnCl_2$, 1.0; $MgCl_2$, 5.0; β-mercaptoaethanol, 0.2; [8-^{14}C]ATP, 0.1, specific activity: 258 counts/min per mμmole; 10 μg DNA; 100 μg of protein. Incubation time 20 min at 37°.

Dose (*krad*)	*mμmoles [8-^{14}C]AMP incorporated*	
	Native DNA	*DNA denatured after irradiation*
0	4.0	25.9
2	5.15	27.8
4	5.6	21.2
6	6.5	21.9
10	5.83	25.4
30	4.42	19.6

contrast to RNA synthesis, where the dose–effect curve shows a continuous decrease of the priming ability[3]. If the DNA is denatured after irradiation, the initial increase is very small.

The priming ability of DNA in the synthesis of poly (A) is dependent on the temperature of incubation (Table II). The increase after γ-irradiation is more pronounced at low temperatures (20°). A similar effect is observed for poly (U) synthesis, but not for RNA synthesis. The inhibition of poly (A) synthesis on UV-DNA, however, is independent of the incubation temperature. These results are in agreement with similar experiments by MEHROTHRA AND KHORANA[7] on thymidine polynucleotides.

Abbreviations: poly (A), polyadenylic acid; poly (U), polyuridylic acid.

TABLE II

TEMPERATURE DEPENDENCE OF THE SYNTHESIS OF POLY (A), POLY (U), AND RNA IN THE PRESENCE OF UNTREATED, γ-IRRADIATED AND ULTRAVIOLET IRRADIATED DNA

The incubation mixture was the same as in Table I, except for 30-min incubation, 20 μg DNA and temperature as indicated. For poly (A) synthesis specific activity of [8-^{14}C]ATP: 324 counts/min per mμmole, for poly (U) synthesis specific activity of [2-^{14}C]UTP: 90 counts/min per mμmole, for RNA synthesis specific activity of [8-^{14}C]ATP: 305 counts/min per mμmole.

		mμmoles nucleotide incorporated						
		15°	*20°*	*25°*	*30°*	*37°*	*40°*	*45°*
Poly (A)synthesis	Untreated DNA	1.5	2.6	4.9	8.2	10.1	13.1	12.0
	γ-DNA	3.7	9.2	10.5	11.4	11.9	12.0	12.9
	UV-DNA	0.7	1.1	1.5	2.0	2.5	2.6	2.8
Poly (U)synthesis	Untreated DNA		0.5	0.9		2.0		0.9
	γ-DNA		1.3	1.5		1.3		0.7
RNA synthesis	Untreated DNA	3.6	4.7	5.6	7.3	7.3	7.2	5.5
	γ-DNA	0.4	0.6	0.7	0.7	0.7	0.9	0.7
	UV-DNA	<0.1	<0.1	<0.1	<0.1	0.1	0.3	0.2

TABLE III

EFFECT OF ULTRAVIOLET IRRADIATED DNA ON THE POLY (A) SYNTHESIS ON UNTREATED DNA

The incubation mixture was the same as in Table I except for 10 μg irradiated and untreated DNA each.

DNA samples in the incubation mixture	*mμmoles [8-^{14}C]AMP incorporated*
Untreated DNA alone	10.4
UV-DNA alone	3.1
Untreated DNA, UV-DNA simultaneously	5.6
Untreated DNA, UV-DNA after 40 sec	7.9
Untreated DNA, UV-DNA after 120 sec	8.6
Untreated DNA, UV-DNA after 210 sec	10.5
Untreated DNA, UV-DNA after 300 sec	10.6

UV-DNA, as well as γ-DNA, is able to inhibit RNA synthesis on untreated DNA[3,4]. The same phenomenon was found for poly (A) synthesis (Table III). Simultaneous addition of 10 μg UV-DNA to the incubation mixture with 10 μg untreated DNA lowers the [8-^{14}C]AMP incorporation by half. When the UV-DNA was added after the synthesis had started, this inhibitory effect was reduced. Addition after 210 sec no longer had any effect. This shows that the enzyme is bound to the untreated DNA within 3.5 min and cannot be removed by the UV-DNA. Corresponding results were found in the case of RNA synthesis[3,4].

The kinetics of poly (A) synthesis under certain conditions is shown in Fig. 1. On UV-DNA, the rate of synthesis is low; addition of untreated DNA 2 min after the synthesis has started leads to a small increase in the synthesis (Fig. 1A). UV-DNA, added 2 min after the start of the synthesis with untreated DNA, reduces the synthetic rate. This shows that there is a competition for the polymerase between the two DNA samples. A corresponding observation was made on γ-DNA (Fig. 1B). Here, irradiated DNA has the higher priming ability.

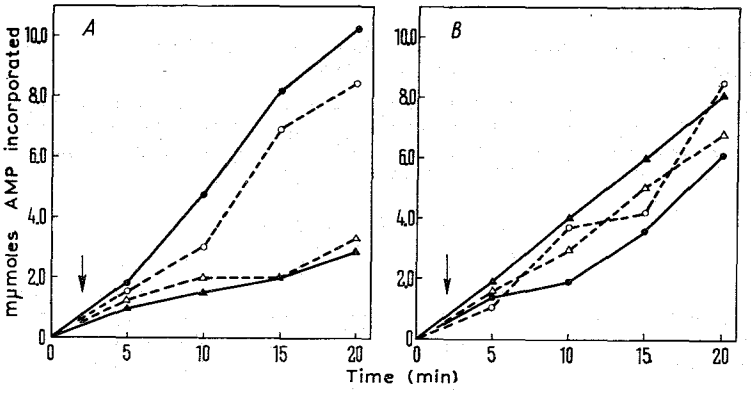

Fig. 1. Kinetics of poly (A) synthesis in the presence of untreated and irradiated DNA. A. Ultraviolet irradiation. B. γ-Irradiation. ●, untreated DNA; ▲, irradiated DNA; ○, untreated DNA, irradiated DNA after 2 min; △, irradiated DNA, untreated DNA after 2 min. Addition of the second DNA sample to the incubation mixture is indicated by arrow. The incubation mixture (1.50 ml) contained in μmoles: Tris buffer (ph, 7.9), 60; $MgCl_2$, 30; $MnCl_2$, 6; β-mercaptoethanol, 1.2; [8-^{14}C]ATP, 0.6, specific activity: 197 counts/min per mμmole; 60 μg of each DNA sample; 600 μg protein. At the times indicated, 0.25 ml of the incubation mixture were removed and the incorporation of [8-^{14}C]AMP determined in the usual way.

The increase of poly (A) synthesis after γ-irradiation may be explained by the fact that ionizing radiation denatures the DNA molecules apart from its effect on the phosphodiester bonds or on the base moieties[8,9]. As only the denatured regions of the DNA molecule act as primer for homopolymer synthesis[10], the priming ability increases in a manner corresponding to the radiation-induced denaturation. The decrease at higher doses may be due to destruction of the nucleotide bases.

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