IN VITRO DNA SYNTHESIS IN THE PRESENCE OF ANTIBODY TO HOMOGENEOUS E. COLI DNA POLYMERASE*

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Summary: After the addition of antiserum against E. coli DNA polymerase to soluble extracts of the DNA polymerase-defective mutant (Pol A_1^-) or the parent strain a DNA synthesizing activity is still present. The antiserum-resistant activity in both the mutant and parent strain has similar requirements: four deoxynucleoside triphosphates, Mg^{++}, and a double-stranded DNA primer. The rates of heat denaturation and the effects of the addition of ethanol and potassium chloride are similar for the antiserum-resistant activities, and differ from the antiserum-sensitive activity of the parent strain and homogeneous E. coli DNA polymerase. Thus, the presence of an additional soluble DNA polymerizing enzyme in E. coli is indicated.

Recent investigations (1-4) have shown that a DNA synthesizing activity is present in the DNA polymerase-defective mutant (Pol A_1^-) (5). If this activity is required for replication it should also be present in the parent strain. We find this to be so and that this new DNA synthesizing activity differs from that of E. coli DNA polymerase.

MATERIALS AND METHODS:

E. coli strains: The polymerase-defective mutant, P 3478 (Pol A_1^-) (5) and the parent strain W 3110 (Pol^+) were gifts from Dr. F. Frankel, and originated in the laboratory of Dr. J. Cairns. E. coli DNA polymerase was purified by the method of Jovin et al. (6), except that the final fraction was subjected to a second chromatography on phosphocellulose before it was

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judged to be homogeneous by polyacrylamide electrophoresis. Anti-E. coli DNA polymerase antiserum was made by injecting a rabbit with 1 mg of the purified enzyme in 2 ml of Freund's complete adjuvant. Two weeks later the animal was injected again and after six weeks it was bled. "Activated" calf thymus DNA was prepared by digesting DNA with pancreatic deoxyribonuclease until maximal priming for sea urchin nuclear DNA polymerase was achieved (7). Thymidine triphosphate-[α-^{32}P] and TTP-^{3}H were obtained from International Chemical and Nuclear Corporation and Schwarz BioResearch, respectively.

Soluble extracts were obtained from the bacteria in exponential growth phase after sonicating at 0°C and freezing and thawing three times in 10 volumes of solution D (20% glycerol containing 0.02 M Tris-maleate buffer (pH 7.8), 4 mM reduced glutathione, and 0.4 mM potassium EDTA). The insoluble material was then removed by sequential centrifugation at 20,000 x g for 30 min. and 100,000 x g for one hour. The clear supernatant was assayed directly or frozen at -70°C. DNA polymerase activity was determined in a reaction mixture (0.3 ml) which contained 25 µmoles Tris-maleate buffer (pH 7.8), 1.8 µmoles $MgCl_2$, 1.0 µmole KCl, 0.3 µmole β-mercaptoethanol, 35 mµmoles dATP, dCTP, dGTP; and 10 mµmoles [α-^{32}P]-dTTP or ^{3}H-dTTP (about 4 x 10^5 dpm); 354 mµmoles "maximally activated" calf thymus DNA (7) and 25 µl of the enzyme preparation. Incubation was for 5 minutes at 37°C and incorporation into an acid-insoluble precipitate was determined (7). The large quantity of primer and short incubation were necessitated by the large amount of deoxyribonuclease activity in Pol$^+$ and Pol A_1^-. Pol A_1^- has 1.5 to 2.3 times the amount of endonuclease activity in Pol$^+$ under the conditions of the polymerase assay. When added, antiserum was preincubated with the enzyme for 10 minutes at 37°C prior to assaying for polymerase.

RESULTS:

Analysis of the polymerase-defective mutant (Pol A_1^-) for a soluble DNA synthesizing system reveals the presence of such an activity in amounts corresponding to 2-15% of that in the parent strain (Pol$^+$). However, the

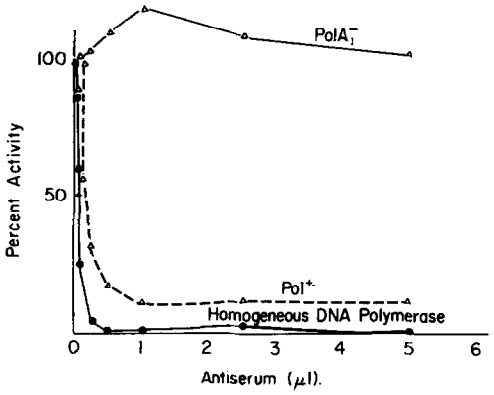

Fig. 1. Effect of anti-E. coli DNA polymerase antiserum on in vitro DNA synthesis. The indicated amounts of antiserum were preincubated with extracts of Pol A_1^-, Pol^+ and homogeneous E. coli DNA polymerase, containing 155, 27 and 0.0015 μg protein, respectively; one hundred percent represents 21, 34, and 14 pmoles $dTM^{32}P$ incorporated, respectively.

activity present in the mutant is entirely resistant to inhibition by anti-DNA polymerase antiserum (Fig. 1). Titration of the soluble supernatant fraction from the parent strain with this antiserum reveals that a significant amount of the total polymerase activity is not inhibited (2 to 14% in different experiments). Ninety-nine percent of the activity of purified E. coli DNA polymerase can be inhibited with as little as 0.5 μl of anti-DNA polymerase antiserum (Fig. 1). The antiserum did not cross-react with partially purified human DNA polymerase (8), purified sea urchin nuclear DNA polymerase (7) and E. coli exonuclease III (6) as measured by immunodiffusion, immunoelectrophoresis and enzymatic activity.

When Pol A_1^- was disrupted and centrifuged through sucrose density gradients (Fig. 2A), all detectable polymerase activity was resistant to anti-DNA polymerase antiserum. In other experiments, controlled digestion of unfractionated extracts of Pol A_1^- with lipase, phospholipase, RNase, papain, pronase, trypsin, as well as disruption by Triton-X, prolonged sonication, or repeated freezing and thawing failed to reveal the presence of an antiserum-susceptible polymerase activity. Analysis of the parent strain (Fig. 2B) shows that most of the polymerase activity can be inhibited with antiserum; yet, a significant amount is resistant.

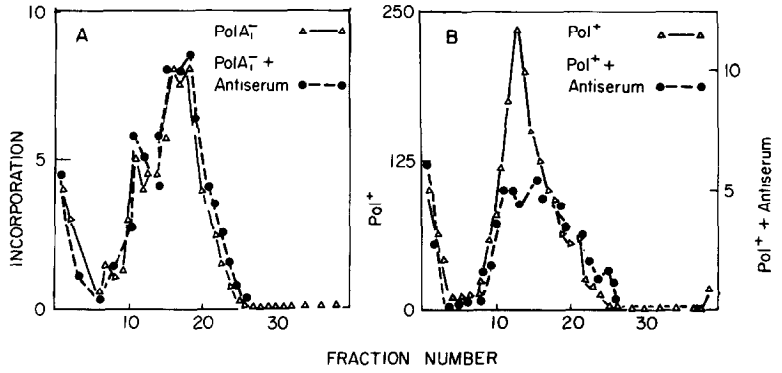

Fig. 2. Sucrose density gradient centrifugation of soluble extracts of Pol$^+$ and Pol A$_1^-$. Bacteria were disrupted in 10 volumes of 0.02 M Tris-maleate buffer (pH 7.5), 0.01 M β-mercaptoethanol and 0.5 ml of this homogenate were immediately layered over 12.5 ml of a 5-20% linear sucrose gradient containing the above buffer. Centrifugation was for 20 hrs. at 40,000 rpm in the Spinco SW 40 Ti rotor. Polymerase activity was determined on 0.1 ml of each fraction after preincubation with or without 2 µl of antiserum. Incubation was for 5 min. at 37°C and incorporation is in µµmoles dTMP-^{3}H.

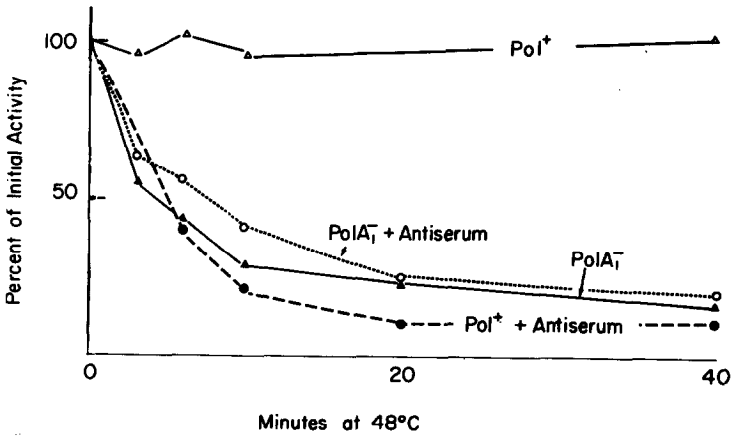

Fig. 3. Loss of enzymatic activity upon heating. Extracts of Pol$^+$ (5 µl) and Pol A$_1^-$ (50 µl) were preincubated with and without antiserum (2 µl), heated as indicated and then assayed for polymerase activity. One hundred percent represents 42 and 34 µµmoles dTM^{32}P incorporated for Pol$^+$ and Pol A$_1^-$.

The requirements for the incorporation of dTTP into DNA by extracts from Pol A$_1^-$ and Pol$^+$ in the presence and absence of antiserum are shown in Table 1. The antiserum-resistant activity is dependent on Mg^{++}, four deoxynucleoside triphosphates, and double-stranded DNA, consistent with its functioning as a

TABLE 1. REQUIREMENTS FOR DEOXYNUCLEOTIDE INCORPORATION INTO DNA.

Reaction Mixture	dTMP-^{3}H Incorporation (mμmoles/5 min)				
	Homogeneous DNA Polymerase (10 μμg)	Pol^+ (58 μg)	Pol^+ (58 μg) + Antiserum (2 μl)	$Pol\ A_1^-$ (73 μg)	$Pol\ A_1^-$ (73 μg) + Antiserum (2 μl)
Complete	98	99	12	15	15
+ Pancreatic Deoxyribonuclease (25 μg)	< 1	< 1	< 1	< 1	< 1
+ KCl (0.1 M)	125	170	11	8	6
+ KCl)0.33 M)	128	135	3	1	1
+ Ethanol (8%)	80	83	21	24	35
+ Ethanol (20%)	47	55	22	23	28
- $MgCl_2$	< 1	3	< 1	< 1	< 1
- dATP	4	12	4	4	4
- dGTP, dATP	< 1	< 1	2	2	2
- dGTP, dCTP, dATP	< 1	3	1	< 1	1
- "Act." DNA	< 1	10	1	< 1	< 1
" " + Denatured "Act." DNA	44	61	1	1	< 1
" " + Calf Thymus Native DNA	8	11	6	6	6
" " + Calf Thymus Denatured DNA	2	8	< 1	1	1
" " + Poly dAT	254	70	8	6	6

Polymerase activity was determined as in "Methods". Deletion experiments were carried out after dialysis of the enzyme preparations against 500 volumes of solution D for 12 hours.

DNA polymerase. Ethanol (8-20%) inhibits Pol^+ activity and the purified enzyme, while it stimulates the antiserum-resistant activity in both the Pol A_1^- and Pol^+. Potassium chloride (0.1-0.33 M) has the opposite effect to ethanol. Moreover, the polymerase activity present in Pol A_1^- is labile at 48°C; its lability is not significantly affected by the presence of anti-DNA polymerase antiserum (Fig. 3). In contrast the activity present in Pol^+ is not altered by this treatment; yet, in the presence of excess antiserum the remaining activity is denatured at the same rate as that present in the mutant, Pol A_1^-.

DISCUSSION:

Our results indicate the presence of a soluble DNA synthesizing activity in the polymerase-deficient mutant (Pol A_1^-). This activity is resistant to anti-*E. coli* DNA polymerase antiserum. Studies on DNA synthesis in membrane-associated fractions of Pol A_1^- utilizing endogenous DNA as a template (1, 2) are difficult to compare with the soluble activity we describe. The results reported by T. Kornberg and M. Gefter (4) on the polymerase activity present in the mutant are in accord with those presented in this communication.

We also find a DNA synthesizing activity in the parent strain which is not inhibited by anti-*E. coli* DNA polymerase antiserum. The reaction catalyzed by the antiserum-resistant activities in the Pol^+ and Pol A_1^- appear similar and typical of a DNA polymerizing system. However, they differ from the classical *E. coli* DNA polymerase in their resistance to the antiserum, activation by ethanol and inhibition by KCl, as well as denaturation by heat. The presence of this polymerizing activity in Pol^+ indicates that the activity present in Pol A_1^- is not an altered polymerase and points to the existence of a second DNA polymerizing system in *E. coli*.

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Errata

We regret the inclusion of a misleading statement in our paper in this journal [R. Wykle and F. Snyder, Biochem. Biophys. Res. Commun., 37, 658 (1969)] and suggest the replacement of the second sentence in the last paragraph with the following: "Hajra [Biochem. Biophys. Res. Commun., 37, 486 (1969)] has independently also shown that DHAP rather than glyceraldehyde-3-phosphate is the precursor of the glyceryl moiety of the alkyl ethers, using liver mitochondria and brain microsomes."

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F. Snyder

Volume 41, No. 3 (1970), in the Communication "Glutamine Synthetase Deadenylylation: A Phosphorolytic Reaction Yilding ADP as Nucleotide Product" by Wayne B. Anderson and E. R. Stadtman, pp.704-709:

Table I, page 707, Experiment B, the isotope in the product AMP should read 263 cpm, not 2630 cpm.