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Enzymatic Synthesis of Deoxyribonucleic Acid

IX. THE POLYMERASE FORMED AFTER T2 BACTERIOPHAGE INFECTION OF *ESCHERICHIA COLI*: A NEW ENZYME*†

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The synthesis of T2 bacteriophage deoxyribonucleic acid is now known to depend on the development of a series of new enzymes which are undetectable in uninfected *Escherichia coli* (2–7). An augmented rate of deoxyribonucleic acid synthesis also occurs (8) which is accompanied by an approximately 10-fold increase in polymerase activity in extracts of these cells, as compared with extracts of uninfected cells (3). In order to observe this increase in polymerase activity, it was essential to assay the enzyme with a *heated* deoxyribonucleic acid primer; the requirement for a *heated* primer is not demonstrable for the polymerase activity in extracts of uninfected cells. An important question regarding the augmented polymerase level was whether it was due to the increased production of the pre-existing *E. coli* polymerase or to the synthesis of a totally new phage-directed enzyme. Koerner, Smith, and Buchanan (6) have also considered this question.

Although it could have been assumed that there was an increase in production of the host polymerase rather than the introduction of information for the synthesis of a totally new enzyme, several facts argued against this. First, there was the novel requirement for a heated deoxyribonucleic acid primer in order to observe the increased polymerase levels (3). Secondly, there was the observation of Bessman and Van Bibber (9) that the 10- to 20-fold elevated level of deoxyguanylate kinase in phage-infected cells does not show the potassium requirement demonstrated for the kinase of the uninfected cells. Finally, it appears that host cell protein synthesis ceases upon infection (10–12), and there is no mechanism at hand to explain how the invading phage deoxyribonucleic acid selects the production, and at an augmented level, of certain kinases and polymerase of the many hundreds of proteins normally produced by the host cell.

Bello, Van Bibber, and Bessman (13) have now shown that the deoxyguanylate kinase of infected cells is physically distinct and separable from the host cell enzyme, and we have made a similar observation for the polymerase. The present report describes the purification and properties of the new polymerase of T2-infected *Escherichia coli* (T2 polymerase) and demonstrates by several different criteria that the polymerase of uninfected *E. coli* (*E. coli* polymerase) and the T2 polymerase are distinct and different enzymes.

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† A preliminary account of this work has appeared (1).

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EXPERIMENTAL PROCEDURE

Materials

Uniformly labeled C^{14}-5′-deoxyguanylate was obtained by enzymatic hydrolysis of DNA from a Chromatium species grown on $C^{14}O_2$ as the sole carbon source (14). Deoxyribonucleoside triphosphates were prepared as described previously (15). DEAE-cellulose and Whatman phosphocellulose (P-70) were products of Brown Company and W. and R. Ralston, Ltd., respectively. Streptomycin sulfate was kindly donated by Merck, Sharp and Dohme Company. Protamine sulfate was obtained from Eli Lilly and Company. Crystallized bovine plasma albumin was obtained from Armour and Company.

Calf thymus DNA was isolated according to Kay, Simmons, and Dounce (16), and salmon sperm DNA, by essentially the same procedure. *E. coli* and bacteriophage T2 DNA's were prepared as described previously (15). DNA from bacteriophage ΦX 174 was kindly furnished by Dr. R. L. Sinsheimer.

"Activated" calf thymus DNA was prepared by exposing the DNA (0.25 mg) to 5×10^{-4} μg of crystalline pancreatic DNase (Worthington Biochemical Corporation) in a solution of 1.0 ml containing 0.5 mg of bovine serum albumin, 5 μmoles of $MgCl_2$, and 50 μmoles of Tris buffer, pH 7.5. After 15 minutes at 37°, the solution was heated for 5 minutes at 77° and then immediately cooled in an ice bath. This DNase treatment improved the DNA as a primer for *E. coli* polymerase, but was not extensive enough to produce acid-soluble nucleotides.

Heated DNA samples were prepared by keeping a solution of DNA (1 μmole per ml in 0.02 M Tris buffer, pH 7.5, and 0.02 M NaCl) at 100° for 4 minutes and then immediately cooling it in an ice water bath. For the net synthesis experiments, the DNA was heated at 100° for 15 minutes and quickly cooled.

Concentrations of DNA are expressed as equivalents of nucleotide phosphorus.

The *E. coli* polymerase was prepared by a modification of the original method (15). The cell extract was prepared by disruption with glass beads in a Waring Blendor, and the activity was then precipitated with streptomycin sulfate. After treatment with pancreatic DNase and fractionation with ammonium sulfate, the enzyme was further purified on DEAE-cellulose and phosphocellulose columns. The specific activity of the phosphocellulose fraction was approximately 1300 and represented a 2600-fold purification of the enzyme from the crude extract. The enzyme was assayed with C^{14}-dGTP as the labeled substrate and "activated" calf thymus DNA as the primer. Publication

TABLE I

Purification of T2 polymerase

Fraction No.	Step	Units		Protein	Specific activity
		Per ml	Total		
				mg/ml	*units/mg protein*
I	Cell extract	41.7	20,850	15.8	2.6
II	Streptomycin	8.5	14,280	2.18	3.9
III	Protamine	9.8	6,272	0.30	32.7
IV	Ammonium sulfate	213	5,325	4.08	52.2
V	Dialysis	160	4,000	3.57	44.8
VI	DEAE-cellulose*	43.9	1,866	0.12	366
VII	Phosphocellulose*	91.4	1,554	0.058	1576†

* These steps were performed many times on a smaller scale (see text), and these values are calculated for the large scale procedure.

† The specific activity of Fraction VII has varied from 1000 to 2200, depending on the particular lot of infected cells used as the source of the enzyme.

of the detailed procedure awaits completion of current attempts to purify this enzyme further.

Antiserum to *E. coli* polymerase was prepared by treating a rabbit with an injection of 2 ml of an emulsion containing equal volumes of *E. coli* polymerase (12.9 mg of the DEAE fraction) and Freund's adjuvant prepared in the VirTis homogenizer. The injection was repeated after 6 weeks.

Antiserum to T2 polymerase was prepared in a similar manner with 1.4 mg of an ammonium sulfate concentrate of T2 polymerase Fraction VI. After 3 weeks, a second injection, representing 2 mg of the same T2 polymerase preparation, was given.

Methods

Assay of T2 Polymerase—The assay measures the conversion of C^{14}- or P^{32}-labeled deoxynucleoside triphosphates into an acid-insoluble product. The incubation mixture (0.3 ml) contained 20 μmoles of Tris buffer, pH 8.6, 2 μmoles of $MgCl_2$, 3 μmoles of 2-mercaptoethanol, 2 mμmoles of Sodium Versenate, pH 6.8, 60 mμmoles of heated salmon sperm DNA, 10 mμmoles each of dHMCTP,[1] dATP, dTTP, and C^{14}-dGTP (2×10^6 c.p.m. per μmole), and 0.01 to 0.1 unit of enzyme. Enzyme dilutions were made in 0.05 M Tris buffer, pH 7.5, containing 0.01 M 2-mercaptoethanol and bovine serum albumin (1 mg per ml). The incubation period was 30 minutes at 37°. The reaction was stopped, and the acid-insoluble radioactivity was determined as described earlier for a glucosyl transferase assay (4).

The radioactivity made acid-insoluble was proportional to the amount of enzyme added; thus, 0.01, 0.02, and 0.04 ml of a phosphocellulose fraction (diluted 1:100) resulted in the appearance of 388, 807, and 1611 c.p.m., respectively, in the acid-insoluble fraction.

A unit of enzyme is defined as the amount catalyzing the incorporation of 10 mμmoles of the labeled deoxynucleotide into the acid-insoluble product during the 30-minute incubation period. Specific activity is expressed as units per milligram of protein.

Protein was determined by the method of Lowry *et al.* (17).

[1] The abbreviations used are: dHMCTP, deoxyhydroxymethylcytidine triphosphate; HMC, 5-hydroxymethylcytidine; d-AT copolymer, copolymer of deoxyadenylate and deoxythymidylate.

Enzyme fractions containing interfering materials such as streptomycin or 2-mercaptoethanol were first precipitated in the cold with trichloroacetic acid. Deoxypentose was determined by the diphenylamine reaction of Dische (18). All optical measurements in the ultraviolet region were made with the Zeiss PMQ II spectrophotometer.

RESULTS

Purification and Properties of T2 Polymerase

The growth and harvest of *E. coli* B infected with $T2r^+$ phage for large scale enzyme preparation have been described previously (reference (3), footnote 19). In the fractionation procedures to be described, the temperature was maintained near 0° except where noted, and centrifugations were at 14,000 × *g* for 20 minutes. The procedure is summarized in Table I.

Preparation of Extract—The first step was to mix 45 g of frozen cells, 150 g of glass beads (Superbrite No. 100), and 50 ml of a buffer solution (A) containing glycylglycine (0.05 M, pH 7.0), Sodium Versenate (0.002 M), and reduced glutathione (0.002 M) for ten 2-minute periods in a 1-quart glass Waring Blendor at two-thirds of maximal speed. The container and its contents were chilled between mixing periods by immersion in an ice water bath until the temperature of the suspension was below 10°. After the last mixing period, 140 ml of Buffer A were added, and mixing was continued at one-fifth of maximal speed for 10 minutes. The glass beads were allowed to settle for 3 minutes, the supernatant fluid was decanted, and the beads were briefly washed with 100 ml of Buffer A. The supernatant and wash fluids were combined and centrifuged, and the resulting supernatant fluid was collected. The procedure was repeated with an additional 45 g of cells, and the supernatant fluids from these two batches were combined to give 500 ml of Fraction I.

Streptomycin Precipitation—To 500 ml of Fraction I were added 420 ml of Buffer A and then, with stirring during a 10-minute period, 276 ml of streptomycin sulfate (5% solution). Stirring was continued for an additional 20 minutes. The precipitate, collected after centrifugation, was homogenized in a Waring Blendor at one-fifth of maximal speed for 30 minutes with 230 ml of a buffer solution (B) containing potassium phosphate (0.05 M, pH 7.4), Sodium Versenate (0.002 M), and reduced glutathione (0.002 M). This suspension was mixed with 596 ml of Buffer B and 840 ml of a solution containing Sodium Versenate (0.002 M) and reduced glutathione (0.002 M) to produce Fraction II.

Protamine Precipitation and Elution—To 1680 ml of Fraction II were added, with stirring, 210 ml of a 1% protamine sulfate solution. The addition required 4 minutes, and stirring was continued for an additional 6 minutes. The suspension was divided into two equal parts, which were treated as separate batches. After centrifugation, the supernatant fluid was discarded, and the precipitate was transferred to a Waring Blendor with 200 ml of potassium phosphate buffer (0.10 M, pH 7.0) containing Sodium Versenate (0.002 M) and reduced glutathione (0.002 M), and homogenized at one-fifth of maximal speed for 10 minutes. An additional 140 ml of this buffer were added, and the mixing was continued for another 5 minutes. After centrifugation, the supernatant fluid was collected. The supernatant fluids from both batches were combined (Fraction III).

Ammonium Sulfate Fractionation—To 640 ml of Fraction III

TABLE II

Requirements of T2 polymerase for deoxynucleotide incorporation into DNA

The complete system was the standard assay mixture (see "Methods") with 0.07 μg of Fraction VII. The labeled substrate was C^{14}-dGTP.

System	C^{14}-deoxynucleotide incorporation
	mμmoles
Complete system	0.71
Omit dATP or dTTP or dHMCTP	<0.03
Omit DNA	<0.01
Omit $MgCl_2$	<0.01
Omit 2-mercaptoethanol	0.32
Omit Sodium Versenate	0.68

were added, with stirring, 131 g of ammonium sulfate. After 15 minutes, the precipitate was removed by centrifugation. To the supernatant fluid an additional 95 g of ammonium sulfate were added, and after 15 minutes the precipitate was collected by centrifugation and dissolved in 25 ml of potassium phosphate buffer (0.025 M, pH 7.4) containing Sodium Versenate (0.002 M) and reduced glutathione (0.002 M) to yield Fraction IV.

Dialysis—Fraction IV, 20 ml, was dialyzed for 4 hours against K_2HPO_4 (0.02 M) containing 2-mercaptoethanol (0.01 M), and the dialysate was immediately used for the preparation of Fraction VI.

DEAE-cellulose Chromatography—A column of DEAE-cellulose (11 × 1.1 cm) was prepared and equilibrated with K_2HPO_4 (0.02 M) containing 2-mercaptoethanol (0.01 M). Fraction V (20 ml) was passed through the column at the rate of 36 ml per hour. The column was then washed with 10 ml of the K_2HPO_4-mercaptoethanol solution. A constant gradient, the limits of which were 0.02 M and 0.30 M potassium phosphate buffer, pH 6.5, was applied. The buffers contained mercaptoethanol (0.01 M), and 150 ml of each buffer were used. Fractions were collected at 12-minute intervals. Elution of 63% of the activity occurred between 8.4 and 14.8 resin-bed volumes of effluent. The peak fraction was eluted in approximately 0.09 M phosphate. The peak fractions having specific activities of 172 to 564 were combined to yield 34 ml of Fraction VI.

Phosphocellulose Chromatography—A column of phosphocellulose (10 × 0.6 cm) was prepared and washed with potassium phosphate buffer (0.02 M, pH 6.5) containing 2-mercaptoethanol (0.01 M) until the pH of the effluent was 6.5. Fraction VI (5.0 ml) was passed through the column at a rate of 0.3 ml per minute. The following potassium phosphate buffers (pH 6.5) containing 2-mercaptoethanol (0.01 M) were used as stepwise eluents: 3 ml of 0.05 M, 6 ml of 0.10 M, 6 ml of 0.15 M, 8 ml of 0.20 M, 2 ml of 0.50 M, and, finally, 2 ml of 0.50 M. Approximately 83 to 99% of the enzyme activity applied to the phosphocellulose column was in the last eluate fraction.

DNase Activities of T2 Polymerase Fractions—The last two fractions in the purification of the T2 polymerase were assayed for endonuclease (19) and phosphodiesterase (20) activities as described by Lehman. The ratios of polymerase to endonuclease (mμmoles nucleotides rendered acid-insoluble-mμmoles rendered acid-soluble) of Fractions VI and VII were 46 and 220, respectively. The polymerase-phosphodiesterase ratios of Fractions VI and VII were 27 and 26, respectively. The endonucleolytic and phosphodiesterase activities were measured on native and heated *E. coli* DNA, respectively.

TABLE III

Specificity of T2 polymerase for deoxynucleoside triphosphates

Control values measured as radioactive deoxynucleotide incorporated into DNA in the presence of dTTP, dATP, dCTP and dGTP, but in the absence of the analogue, were 3.56 mμmoles of C^{14}-dGTP for columns 1, 2, and 3, and 4.17 mμmoles of C^{14}-dATP for column 4. Reaction mixtures were the standard assay system (see "Methods") with 0.92 μg of Fraction VII. Reaction mixtures were incubated at 37° for 60 instead of 30 minutes.

Analogue, used in form of deoxynucleoside triphosphate	Deoxynucleoside triphosphate replaced by analogue			
	dTTP	dATP	dCTP	dGTP
	% of control value			
5-Bromouracil	100	<2	<2	<2
5-Fluorouracil*	9	<2	<2	<2
5-Bromocytosine	<2	<2	104	<2
5-Fluorocytosine†	<2	<2	67	<2
5-Hydroxymethylcytosine	<2	<2	98	<2

* Gift of Dr. C. Heidelberger, University of Wisconsin.

† Gift of Dr. R. Duschinsky, Roche Laboratories, Inc.

TABLE IV

Relative effectiveness of various DNA primers for T2 polymerase

Reaction mixtures contained the standard assay system (see "Methods"), with 17 mμmoles of the DNA primer and 0.14 μg of Fraction VII; C^{14}-dATP was used as the labeled substrate when the d-AT copolymer was the primer. When heated salmon sperm DNA was the primer, 0.75 mμmole of C^{14}-dGTP was incorporated. This value, converted to total DNA on the basis of the base composition of salmon DNA, was set at 100; the other values in the table are expressed on a comparative basis.

Source of DNA	Priming activity	
	Heated DNA	Native DNA
Salmon sperm	100	5
dG-dC Homopolymer*	58	
DNA purified from heated sonicate of T2-infected *E. coli*	57	
Calf thymus	20	0.7
Salmon liver	15	4
ΦX 174 bacteriophage		13
d-AT copolymer†	11	
E. coli	10	0.3
T2 bacteriophage	8	0.8
"Activated" calf thymus	1	

* The preparation of dG-dC homopolymer, a deoxyguanylate homopolymer hydrogen bonded to a deoxycytidylate homopolymer, will be reported by C. M. Radding, J. Josse, and A. Kornberg.

† d-AT copolymer was prepared by the method of Schachman *et al.* (23).

Requirements for Reaction—As with the polymerase of *E. coli* (21), the omission of a single one of the four deoxynucleoside triphosphates or of DNA or $MgCl_2$ reduced the reaction to undetectable levels (Table II). The purified T2 enzyme was about half as active when 2-mercaptoethanol was omitted and was unaffected by the absence of Sodium Versenate; T2 polymerase activity of crude extracts was stimulated about 2-fold by the

presence of Sodium Versenate in the assay mixture. Replacing the $MgCl_2$ by an equimolar amount of $MnCl_2$ reduced the incorporation 90%.

Deoxynucleoside Triphosphate Specificity—With the polymerase of *E. coli*, analogues of the naturally occurring bases serve as substitutes in a manner dictated by the hydrogen-bonding relationships of adenine to thymine and of guanine to cytosine in the Watson and Crick model (22). The same behavior has now been observed in the action of T2 polymerase. With halogen substitutions in place of the methyl group on carbon 5 of dTTP, there is a specific replacement of dTTP; with halogen (and also hydroxymethyl) substitutions on carbon 5 of dCTP, there is a specific replacement of dCTP (Table III).

Relative Effectiveness of Various DNA Primers—DNA's isolated from *E. coli*, bacteriophage T2, and animal tissues supported only 5 to 10% of the polymerase activity when compared with these same DNA's when they had been heated (Table IV). The conditions of heating, *i.e.* 4 minutes a 100° in 0.02 M NaCl-0.02 M Tris, pH 7.5, were sufficient in each case tested to produce an optical density increase after rapid cooling of approximately 30%. The most active of the unheated DNA preparations was the ΦX 174 sample, which has the properties of a single-stranded molecule (24).

Net Synthesis of DNA—With the most highly purified fraction of T2 polymerase and with heated T2 DNA as primer, the amount of DNA synthesized was approximately 14% of the amount of primer added (Fig. 1). Values as high as 35%, however, have been obtained, depending on the enzyme preparation used. Unheated T2 DNA is virtually inactive (less than 0.4%) (Fig. 1). When ΦX DNA or heated salmon sperm DNA was used as primer, the amounts of synthesis were 45 and 60%, respectively. These results are in sharp contrast with the extensive increase (10- to 20-fold) in the DNA enzymatically synthesized with *E. coli* polymerase, with an unheated DNA as primer (21, 25).

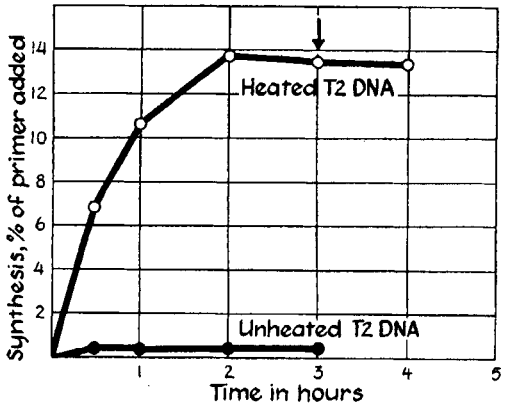

FIG. 1. Net synthesis with T2 DNA as primer. The reaction mixture contained 40 μmoles of Tris buffer, pH 8.6, 4 μmoles of $MgCl_2$, 6 μmoles of 2-mercaptoethanol, 150 mμmoles each of dATP, dCTP, dTTP, and dGTP-C^{14} (4.7×10^5 c.p.m. per μmole, 300 mμmoles of T2 DNA, 0.02 ml of enzyme diluent (see "Methods"), and 2.2 μg of T2 polymerase Fraction VII, in a final volume of 0.60 ml. At the time shown by the *arrow*, an additional 1.1 μg of T2 polymerase were added.

Features Distinguishing E. Coli and T2 Polymerases

Selective Inhibition of Polymerases by Specific Polymerase Antisera—The incubation of 0.1 and 0.2 unit of *E. coli* polymerase with 0.03 ml of rabbit antiserum prepared against purified *E. coli* polymerase results in a virtually complete inhibition (more than 98%) of the enzyme activity. The inhibition is reversed by the addition of increasing amounts of the enzyme (Fig. 2*A*). Over a similar range of concentrations, the purified T2 polymerase is not inhibited (less than 2%) by antiserum to *E. coli* polymerase (Fig. 2*A*).

Conversely, rabbit antiserum prepared against T2 polymerase inhibits T2 polymerase but does not inhibit *E. coli* polymerase (Fig. 2*B*).

Essentially the same inhibitory activity of antiserum to *E. coli* polymerase is seen on crude extracts. The polymerase activity of crude extracts of *E. coli* is inhibited approximately 95%, and that of infected cells less than 20%, by antiserum to *E. coli* polymerase.

Selective Inhibition of T2 Polymerase by p-Chloromercuribenzoate—At levels of *p*-chloromercuribenzoate (1.7×10^{-4} M) which inhibit the T2 polymerase almost completely (more than

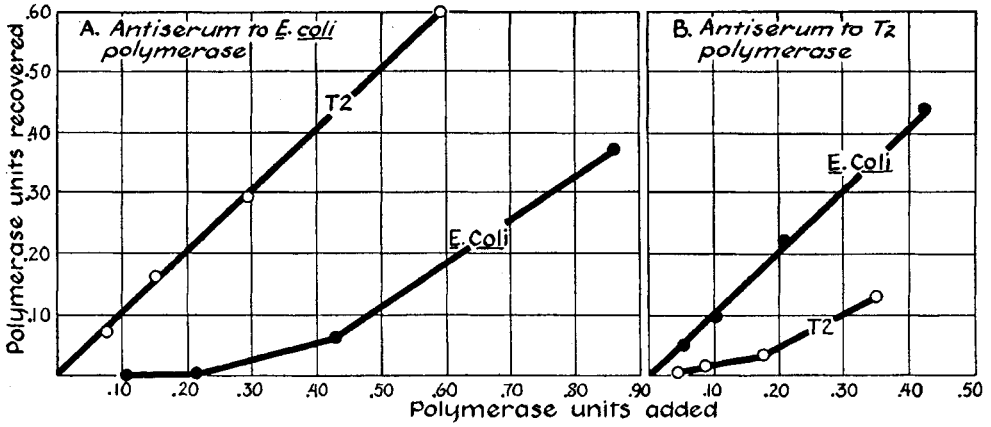

FIG. 2. Selective inhibition of polymerases by specific polymerase antisera. *A*. The reaction mixture (0.20 ml) contained 0.03 ml of antiserum to *E. coli* polymerase, 3 mμmoles of Sodium Versenate, 30 μmoles of NaCl, 30 μmoles of Tris (7.5), and *E. coli* polymerase phosphocellulose fraction or T2 polymerase (Fraction VII). After incubation at 37° for 10 minutes, the mixture was chilled, an aliquot was removed, and the polymerase activity was determined by the standard assay system (see "Methods"). *B*. The reaction mixture and procedure were as in Fig. 2*A* except that 0.04 ml of antiserum to T2 polymerase was used in place of the antiserum to *E. coli* polymerase.

98%), the *E. coli* polymerase retains 73% of its activity (Table V).

Difference in Primer Effectiveness for Two Polymerases—As shown in Table IV, heated salmon sperm DNA is the most active primer of a group of DNA's tested with T2 polymerase, and 100 times more effective than "activated" calf thymus DNA, a highly effective primer for *E. coli* polymerase, as shown by the data described in the legend of Fig. 3. When a mixture of the two polymerases was incubated with heated or unheated DNA as primer, the result was as expected, namely, the sum of the two separate enzyme activities (Table VI). This experiment indicates that if an inhibitor were present in the T2 polymerase preparation to account for its inability to act on native DNA, or if there were a stimulatory factor in the *E. coli* polymerase preparation permitting it to act on native DNA, then these factors are not present in excess.

Chromatographic Separation of Two Polymerases—Partially purified *E. coli* polymerase was eluted from a phosphocellulose column by 0.15 to 0.20 M potassium phosphate (Fig. 3*A*), whereas 0.5 M phosphate was required to elute the T2 polymerase (Fig. 3*B*). When a mixture of the two purified polymerases was chromatographed on phosphocellulose, there were two discrete peaks of activity corresponding to the *E. coli* and T2 polymerases when heated salmon sperm DNA was used as the primer (Fig. 3*C*). Additional evidence that these two peaks represent the two separate enzyme activities was obtained by using a different DNA as primer. When "activated" calf thymus DNA was used as a primer for the assay of the eluted fractions, only the *E. coli* polymerase activity was detected (Fig. 3*D*); as anticipated, the activity of the fractions in the T2 polymerase zone was less than 5% of the activity found when heated salmon sperm DNA was the primer. When aliquots of each of the two peaks were incubated with *E. coli* polymerase antiserum, the enzyme activity in the first peak was inhibited over 90%, whereas the activity in the second peak was not reduced at all.

TABLE V

Inhibition of polymerases by p-chloromercuribenzoate

Reaction mixtures were as in the standard assay system (see "Methods"), with 0.02 μg of T2 polymerase, Fraction VII, and 0.05 μg of *E. coli* polymerase, phosphocellulose fraction, except that Sodium Versenate and 2-mercaptoethanol were omitted. The labeled substrate was C^{14}-dGTP.

p-Chloromercuribenzoate	C^{14}-deoxynucleotide incorporated	
	T2 polymerase	*E. coli* polymerase
$M \times 10^4$	mμmole	
None	0.201	0.597
0.17	0.004	0.435
1.7	0.003	0.429

TABLE VI

Primer differences of T2 and E. coli polymerases

Reaction mixtures were as in the standard assay system (see "Methods"). In Experiment I, 0.09 μg of T2 polymerase, Fraction VII, or 0.10 μg of *E. coli* polymerase, phosphocellulose fraction, was used. In Experiment II, twice the amounts of enzyme were used. The labeled substrate was C^{14}-dGTP.

DNA primer	C^{14}-deoxynucleotide incorporated		
	T2	*E. coli*	T2 + *E. coli*
	mμmoles		
Experiment I:			
Unheated salmon sperm....	0.175	0.995	1.30
Heated salmon sperm.....	0.635	0.492	1.04
Experiment II:			
Unheated salmon sperm....	0.249	2.00	2.11
Heated salmon sperm.....	1.30	1.20	2.84

DISCUSSION

Present knowledge regarding the biochemical changes found in extracts of *E. coli* infected with the virulent T2 bacteriophage suggests the following series of events. Upon entry of the phage DNA, there is an immediate inactivation of the *E. coli* genome. As a result, the bacterial genome no longer supports synthesis of the host proteins, nor does it serve as a template for DNA replication. At the same time that the bacterial genome is eliminated as a functional entity, the invading phage DNA preempts both its phenotypic and genotypic functions. Within 4 minutes after infection, new enzymatic reactions are observed which provide for the synthesis of the distinctive phage DNA. Certain other reactions which were present before infection and are involved in DNA biosynthesis are increased in rate by 10-fold or greater (3, 9, 13); these increased reaction rates are presumably necessary to support the rapid multiplication of the phage. It may be assumed also that the enzymatic machinery of the host cell responsible for energy production and various biosyntheses remains unchanged and serves to support the production of new phages (11).

To account for the novel reactions, synthesis of new enzymes such as hydroxymethylase (2), HMC deoxynucleotide kinase (3, 7), dCDP-dCTPase (3, 5, 6), and DNA glucosyltransferases (4) takes place and is most probably directed by the invading phage DNA. The evidence from the experiments of Bello, Van Bibber, and Bessman with the deoxynucleotide kinases (13) and from our current studies with polymerase indicates that the increased levels of enzymes carrying out reactions already present in the uninfected cell are due also to novel proteins.

The conclusion that the augmented polymerase level of phage T2-infected cells is due to an enzyme distinctly different from the polymerase of uninfected *E. coli* is based on four criteria: (*a*) immunochemical specificity, (*b*) inhibition by *p*-chloromercuribenzoate, (*c*) chromatographic behavior on phosphocellulose, and (*d*) primer requirements. Although the differences in the two polymerases with respect to any one criterion may not be a decisive argument in themselves, the difference in behavior with regard to all four is more convincing. In view of these results, the use of polymerase antiserum can be recommended as a tool for exploring differences between polymerase activities in cells infected with other virulent or temperate phages. The greater sensitivity of the T2 polymerase to inhibition by *p*-chloromercuribenzoate and its stronger affinity for phosphocellulose imply, as does its immunochemical specificity, distinctive differences in protein structure. The basis for the dissimilarity in primer requirement also suggests variations in the structure of the enzymes, but requires further comment.

Activities with the T2 polymerase are 10 to 20 times greater with a DNA sample after it has been denatured by heating. Also, the single-stranded DNA from phage ΦX 174 is an effective primer without heating. These results point to a clear

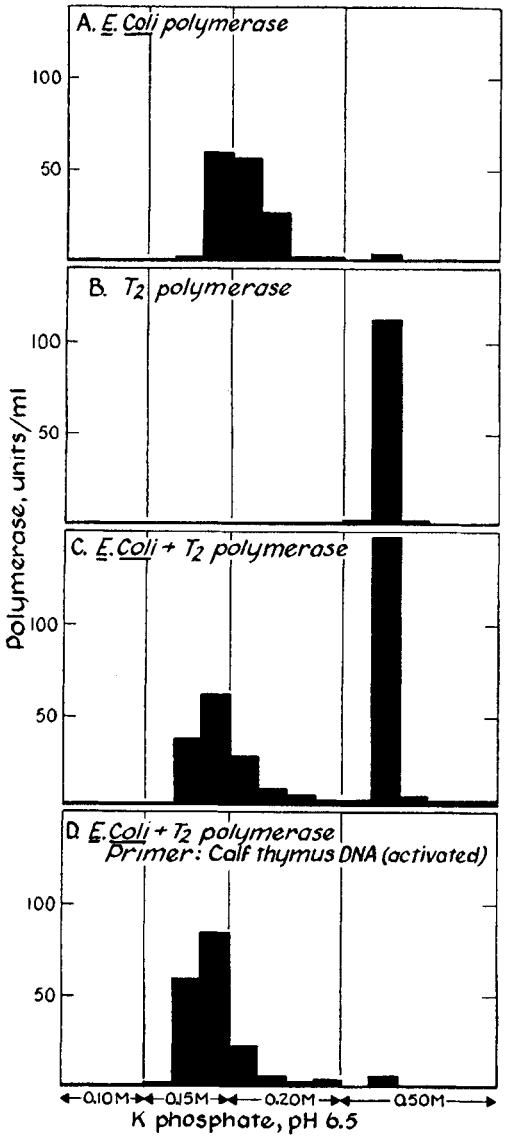

FIG. 3. Chromatographic separation of T2 and *E. coli* polymerases. The preparation of the phosphocellulose columns (10 × 0.6 cm) and the volumes of the eluting buffers are described in the purification procedure. All buffers contained 0.01 M 2-mercaptoethanol. The enzyme fractions applied to the column were adjusted to a phosphate concentration of 0.05 M by either dilution or dialysis. The activities of the eluted fractions were assayed by the standard assay system (see "Methods"), except that either heated salmon sperm DNA or "activated" calf thymus DNA was used as the primer as specified. With *E. coli* polymerase, phosphocellulose fraction, and T2 polymerase (Fraction VI), the mμmoles of C^{14} nucleotide incorporated were 0.334 and 0.314, respectively, when heated salmon sperm DNA was the primer. However, when "activated" calf thymus DNA was the primer, 0.634 and 0.025 mμmoles were incorporated with *E. coli* or T2 polymerase, respectively. *A*. Diluted *E. coli* polymerase (0.5 mg), phosphocellulose fraction, was applied to the column. Of the activity applied, 65% was recovered in the active fractions. Primer: heated salmon sperm DNA. *B*. A dialyzed ammonium sulfate concentrate of T2 polymerase Fraction VI (1.68 mg) was applied to the column. Recovery of the activity was 85% in the active fraction. Primer: heated salmon sperm DNA. *C*. A mixture of *E. coli* polymerase, phosphocellulose fraction, and T2 polymerase Fraction VI described in Fig. 3, *A* and *B* was placed on the column. Of each of the activities applied to the column, 70% was recovered. Primer: heated salmon sperm DNA. *D*. The eluted fractions of the column described in Fig. 3*C* were assayed with "activated" calf thymus DNA (see "Methods").

preference by the T2 polymerase for collapsed or single-stranded DNA. This conclusion is further supported by preliminary investigations of the effects of endonucleolytic digestion on the priming capacity of a DNA sample. Thus, incubation of a T2 DNA sample with small amounts of Lehman's *E. coli* endonuclease increases the subsequent action of the T2 polymerase by a factor of 5; the endonuclease treatment followed by heating at 70° for 5 minutes (by itself ineffective) increases the effectiveness of the T2 primer by a factor of 25. By contrast, the activity of native and denatured DNA samples as primers for *E. coli* polymerase shows relatively small differences. However, it should be noted that some highly purified preparations of *E. coli* polymerase show a slight activity with native DNA which can be greatly enhanced by limited nuclease digestion or heating of the DNA (25). Bollum's findings (26) that calf thymus polymerase preparations, even early in the purification procedure, are far more active with heated DNA than with native DNA is another instance of the phenomena observed with T2 polymerase. Our inability to explain why the T2 polymerase fails to replicate native T2 DNA indicates the gap in our information as to how a native DNA sample is first rendered an active template for replication by any polymerase system. At the core of this unsolved problem is the mechanism which enables the strands of the native DNA molecule to separate sufficiently so that enzymatic replication can be initiated. An understanding of how the phage DNA preempts the functions of the host's genetic material also may hinge on elucidation of this same problem of how double-stranded DNA is readied for its replication.

SUMMARY

The increase in deoxyribonucleic acid polymerase activity after T2 bacteriophage infection of *Escherichia coli* has been shown to be due to the synthesis of a polymerase different from that present in the host cell. This new polymerase, which is induced by T2 infection (T2 polymerase), has been purified approximately 600-fold.

The T2 polymerase has been shown to be different from *E. coli* polymerase by four different criteria: (*a*) a rabbit antiserum prepared against purified *E. coli* polymerase completely inhibits *E. coli* polymerase but does not affect the T2 polymerase; T2 polymerase antiserum inhibits T2 polymerase but not *E. coli* polymerase; (*b*) at levels of *p*-chloromercuribenzoate which inhibit T2 polymerase almost completely (more than 98%), the *E. coli* polymerase retains 73% of its activity; (*c*) heated deoxyribonucleic acid is 10 times more effective than unheated deoxyribonucleic acid as a primer for T2 polymerase but is not a preferred primer for *E. coli* polymerase fractions; (*d*) a mixture of the two enzymes is sharply and quantitatively separated by chromatography on a phosphocellulose column.

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