THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 249, No. 19, Issue of October 10, pp. 6244-6249, 1974
Printed in U.S.A.

A Holoenzyme Form of Deoxyribonucleic Acid Polymerase III

ISOLATION AND PROPERTIES*

(Received for publication, April 22, 1974)

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SUMMARY

A new form of DNA polymerase III (pol III), termed holoenzyme, has been purified to apparent homogeneity from gently lysed *Escherichia coli*. Three forms of pol III have now been recognized on the bases of their distinctive physical characteristics and their capacity to utilize a predominantly single-stranded template: (*a*) pol III, a dimer of 90,000-dalton subunits, which is inactive on single-stranded circular DNA (SS DNA); (*b*) pol III*, a higher polymer of pol III, which is active on SS DNA only in the presence of copolymerase III* (copol III*), a 77,000-dalton polypeptide; and (c) holoenzyme, a 330,000-dalton tetramer composed of the two pol III subunits and two copol III* units, which is distinguished from pol III or pol III* by its activity on SS DNA in the absence of added copol III*. The holoenzyme is dissociated into pol III* and copol III* by chromatography on phosphocellulose; pol III* is converted to pol III by heating, dilution, or aging. Like pol III*, the holoenzyme requires ATP to form an initiation complex with the primer template.

DNA polymerase III (pol III) (1), a product of the *dnaE* gene (2), is inactive in the conversion of ϕX174 and M13 single-stranded viral DNA (SS DNA) to the double-stranded replicative form. However, a more complex form of the enzyme, DNA polymerase III* (pol III*) (3), can catalyze the conversion in the presence of an additional protein, copolymerase III* (copol III*). Starting with an RNA-primed SS DNA[1] template, the first step in this process is the formation of an initiation complex (4) consisting of the primed template, spermidine (or DNA-unwinding protein), ATP, and the two proteins, pol III* and copol III*. In this step, ATP is split into ADP and inorganic phosphate. Once the complex is formed, replication of SS DNA to double-stranded replicative form requires neither ATP nor copol III*. Pol III* cannot be replaced by pol III in this reaction. Pol III* differs from pol III in its physical as well as catalytic properties and appears to be a multimeric form of pol III (3).

* This work was supported in part by grants from the National Institutes of Health and from the National Science Foundation. W. Wickner was supported by a National Cystic Fibrosis Research Foundation fellowship and is currently a Fellow of the Mellon Foundation.

[1] The abbreviations used are: SS DNA, single-stranded circular DNA; pol I, DNA polymerase I; pol III, DNA polymerase III; pol III*, DNA polymerase III*; holoenzyme, DNA polymerase III holoenzyme; copol III*, copolymerase III*.

We have now found a third (and presumably more native) form of pol III. This form, termed pol III holoenzyme, is a tetramer of 330,000 daltons containing the two pol III subunits and two copol III* polypeptides. Replication of SS DNA templates by pol III holoenzyme does not require added copol III* and is similar to that catalyzed by mixtures of pol III* and copol III* in its sensitivity to antibody against copol III* and its dependence on ATP. Phosphocellulose chromatography dissociates the holoenzyme into pol III*, a tetramer of the *dnaE* polypeptide, and copol III* (3). Pol III* is thermosensitive when isolated from *dnaE* mutant cells (3). Pol III* can be converted irreversibly to pol III by aging, dilution, or mild heat treatments.

MATERIALS AND METHODS

Materials—Materials were obtained as before (3, 4). ^{3}H-labeled ϕX174 was a gift of S. Michal Jazwinski of Stanford. Human hemoglobin was prepared by washing packed erythrocytes in 1 M NaCl solution and then lysing them with 5 volumes of water; debris was removed by centrifugation (20 min at 40,000 × *g*) and by filtration through DEAE-cellulose in 0.15 M NaCl. Pol I was the Fraction VII enzyme of Jovin *et al.* (5). RNA polymerase was purified by a minor modification of the method of Babinet (6). Other sources were: rabbit muscle lactic acid dehydrogenase from Sigma, sperm whale myoglobin from Mann, and beef liver catalase from P-L Biochemicals.

Holoenzyme Assay—Holoenzyme was assayed at 30° in a 25-μl reaction mixture containing 3 μl of a deoxynucleoside triphosphate mixture (40 mM $MgCl_2$, 0.15 mM [α-^{32}P]dCTP at 100 cpm per pmole, and 0.4 mM each of dATP, dGTP, and dTTP); 5 μl of assay buffer (10% sucrose, 50 mM Tris·HCl (pH 7.5), 50 mM NaCl, 40 mM dithiothreitol, RNA-primed ϕX174 SS DNA (2.0 nmoles of nucleotide), prepared as described previously (3); 80 μM ATP; 4 mM spermidine·Cl and *Escherichia coli* phospholipids (1 μl of a 4-mg per ml suspension in water). After a 5-min incubation, acid-insoluble ^{32}P-nucleotide was determined by filtration (7). One unit of holoenzyme is defined as the amount catalyzing the incorporation of 1 nmole of nucleotide per min at 30°.

Phospholipid—Phospholipid was extracted from an *E. coli* H560 cell suspension (see below) by the method of Bligh and Dyer (8). After removal of chloroform under a nitrogen stream, the phospholipids were suspended by sonication for 30 s at 0° in 10 mM dithiothreitol. When neutralized with Tris base, the suspension could be stored at 0° for at least 4 months without loss of its ability to support holoenzyme activity.

Glycerol Gradient Sedimentation—Glycerol gradient sedimentation was performed in polyallomer tubes which were boiled for 4 hours in a solution of bovine serum albumin (1 mg per ml) and EDTA (10 mM), washed with water, and then boiled in several changes of 1 mM EDTA. Gradients were composed of 3.6 ml of 35 to 50% glycerol, containing 25 mM imidazole acetate, 20 mM dithiothreitol, and 1 mM EDTA. Sedimentation was at −5° for 9 to 12 hours at 55,000 rpm, in a Beckman SW 56 rotor. Fractions

of 2 drops were collected through a 21-gauge needle which punctured the bottoms of the tubes.

Growth of Cells—E. coli H560 was grown as previously described (3). Cell suspensions (5×10^{10} cells per ml; 0.2 g of wet paste per ml) were neutralized with Tris base just before freezing in liquid nitrogen and storing in 15-ml aliquots at −20°.

RESULTS

Purification of Holoenzyme

Unless noted, all operations were at 0–4° and are summarized in Table I.

Cell Extract—The cell suspension was thawed in a water bath set at 4°. After adjustment of 800 ml of cell suspension to pH 7.5 with solid Tris base, 40 ml of a mixture of 2 M NaCl, 2 mg per ml of lysozyme, and 0.2 M spermidine·Cl were added. The cell suspension was distributed into four 250-ml Sorvall GSA bottles and kept near 0° for 30 min. The bottles were then gently swirled for 4 min in a 37°-bath and centrifuged for 60 min at 12,000 rpm in a Sorvall GSA rotor; the supernatant fluid then was gently decanted: Fraction I (604 ml).

Ammonium Sulfate Fractionation—Finely ground ammonium sulfate (0.226 g per ml) was added to Fraction I over a 2-min period with rapid stirring. Stirring was continued for 20 min and the pH was kept at 7.5 by addition of Tris base. The precipitate was collected by centrifugation (10 min at 10,000 × *g*) and resuspended with a glass-Teflon homogenizer in 75 ml of a solution containing 0.2 g of ammonium sulfate per ml of Buffer A (20% glycerol, 50 mM Tris·HCl (pH 7.5), 0.1 M NaCl, 1 mM EDTA, 1 mM dithiothreitol). The suspension was centrifuged at once for 10 min at 16,000 × *g*. Precipitates were washed three times by suspension in 12 ml of Buffer A containing 0.15, then 0.14, and then 0.13 g of ammonium sulfate per ml. Each suspension was performed with a glass-Teflon homogenizer and the precipitate was collected each time by centrifugation for 5 min at 40,000 × *g*. The washed precipitate was dissolved in 3 ml of Buffer A and desalted by filtration through a column (2 × 20 cm) of Sephadex G-25 equilibrated with Buffer C (30% glycerol, 50 mM Tris·HCl (pH 7.5), 10 mM dithiothreitol, 1 mM EDTA): Fraction II (10.6 ml).

TABLE I

Purification of pol III holoenzyme

Fraction	Total units	Specific activity
		units/mg
I. Supernatant	423	0.028
II. Ammonium sulfate	320	14
III. DEAE-cellulose	162	49
IV. Gel filtration	98	240
V. Glycerol gradient	48	440

DEAE-cellulose—Fraction II was applied to a column (2 × 6 cm) of DEAE-cellulose equilibrated with Buffer C, and the holoenzyme was eluted with a linear NaCl gradient (160 ml, 0 to 0.3 M): Fraction III (14.5 ml) (Fig. 1*A*, *Fractions 19* and *20*).

Gel Filtration—Fraction III was mixed with an equal volume of saturated, neutralized ammonium sulfate. After 30 min, the precipitate was collected by centrifugation for 20 min at 45,000 × *g*, dissolved in 0.5 ml of Buffer A, and applied to a Bio-Gel A-5m column (1 × 13 cm) in Buffer A. Holoenzyme emerged as a peak retarded by inclusion in the gel: Fraction IV (1.8 ml) (Fig. 1*B*, *Fractions 15* to *26*).

Glycerol Gradient Sedimentation—Fraction IV was mixed with an equal volume of saturated, neutralized ammonium sulfate. After 30 min, the precipitate was collected by centrifugation for 20 min at 45,000 × *g*, dissolved in 50 μl of buffer (10% glycerol, 50 mM Tris·HCl (pH 7.5), 1 mM EDTA, 20 mM dithiothreitol), and layered on a 3.6-ml glycerol gradient and sedimented (see "Materials and Methods"): Fraction V (0.4 ml) (Fig. 1*C*, *Fractions 16* to *22*).

For best results, the entire procedure should be performed without interruption, and it can be completed within 24 hours. Holoenzyme, purified 16,000-fold by this procedure, is usually stable for 2 to 3 weeks at 0°.

Phosphocellulose chromatography, which separates holoenzyme into pol III* and copol III*, was avoided in the isolation of holoenzyme. When fractions at several stages of purification were assayed in the presence of pol III* or copol III* (Fig. 1), there was no stimulation of holoenzyme activity. The failure to detect free pol III* or copol III* activities could not be attributed to co-migration of pol III* and copol III*, inasmuch as they sediment more slowly than holoenzyme in glycerol gradients (see Figs. 4 to 6). We have found no indication of free pol III* or copol III* in these lysates (Fraction I); these activities appear when holoenzyme is chromatographed on phosphocellulose (3).

Subunit Structure of Holoenzyme

Holoenzyme (10 μg) was heated in 1% sodium dodecyl sulfate-1% β-mercaptoethanol and subjected to acrylamide gel electro-

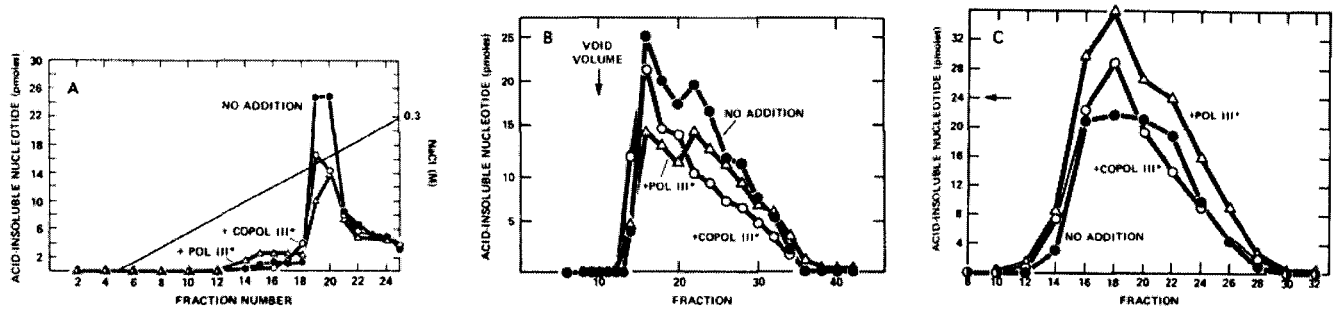

FIG. 1. Chromatography, filtration, and sedimentation of pol III holoenzyme. *A*, DEAE-cellulose. Fraction II (5 ml, 21 mg, 350 units) was applied to a DEAE-cellulose column (2 × 6 cm) in Buffer C and eluted with a linear salt gradient (150 ml, 0 to 0.3 M NaCl, 6-ml fractions). *B*, gel filtration. Fraction III (24 ml, 3 mg, 180 units) was concentrated to 1 ml with ammonium sulfate (see "Materials and Methods") and applied to a column (1.5 × 12 cm) of Bio-Gel A-5m equilibrated with Buffer A. *C*, glycerol gradient sedimentation. Fraction IV (8.8 ml, 0.4 mg, 120 units) was concentrated with ammonium sulfate and sedimented in a glycerol gradient (see "Materials and Methods"). Aliquots (2 μl) of each fraction were assayed for holoenzyme without further addition or with addition of either 15 units of copol III* or 0.5 unit of pol III*. The direction of sedimentation is indicated by an *arrow* in this and subsequent figures.

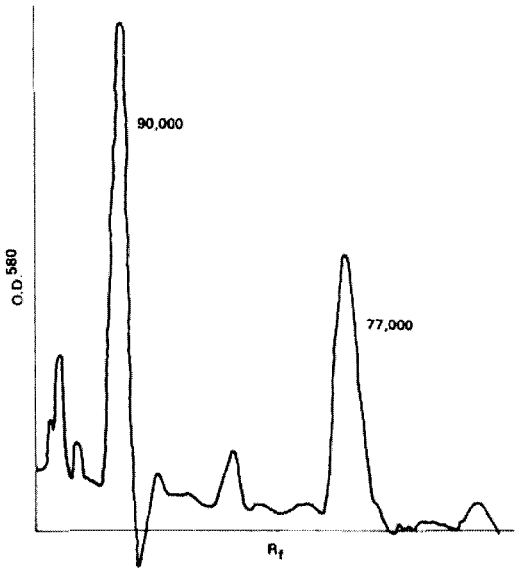

FIG. 2. Sodium dodecyl sulfate gel electrophoresis of holoenzyme. Fraction V holoenzyme (10 μg in 100 μl) was heated for 30 min at 45° with 1% sodium dodecyl sulfate-1% β-mercaptoethanol. Electrophoresis was in the Tris system of Jovin *et al.* (9) at 4°, 200 volts, with 0.1% sodium dodecylsulfate in the gel and in the reservoir buffers. Gels contained 12% acrylamide and 0.25% bisacrylamide and were stained with Coomassie blue by the method of Weber and Osborn (10). Destained gels were scanned at 580 nm in a Gilford recording spectrophotomer.

TABLE II

Ratio of subunits in holoenzyme

Sodium dodecyl sulfate gel electrophoresis of holoenzyme was as in Fig. 3. Tracings of the 90,000- and 77,000-dalton peaks were cut out and weighed; the pol III peaks in Experiments 1, 2, and 3 weighed 18, 54, and 63 mg, respectively. Weight ratios were multiplied by 90,000/77,000 to give molar ratios.

Experiment No.	Holoenzyme	Copol III*:pol III polypeptides	
		Weight ratio	Molar ratio
	μg		
1	2	0.89	1.04
2	4	1.00	1.17
3	6	0.90	1.05

phoresis (Fig. 2). Two prominent protein bands were seen at 90,000 and 77,000 daltons. Similar gels were loaded with 2, 4, and 6 μg of the purified holoenzyme. After staining the protein with Coomassie blue, gels were scanned at 580 nm and the tracings of the 90,000- and 77,000-dalton peaks were cut out and weighed. When the weight ratios of the peaks were corrected for molecular weight, an equimolar ratio for two polypeptides was observed (Table II). The 90,000-dalton polypeptide is presumed to be the pol III (*dna*E) polypeptide (see below and Refs. 2 and 3) and the 77,000-dalton polypeptide, copol III* (see Fig. 9).

To determine the molecular weight of holoenzyme, it was filtered through a Bio-Gel A-5m column and sedimented in glycerol gradients. Both gel filtration (Fig. 3) and glycerol gradient sedimentation (Fig. 4) showed that holoenzyme has a molecular weight greater than that of catalase (247,000) but less than that of RNA polymerase (490,000). On the basis of these data and the equimolar proportions of the 90,000- and 77,000-dalton polypeptides (Table II), the holoenzyme is presumed to be larger than a dimer (167,000 daltons) and smaller than a hexamer (501,000 daltons), and therefore most probably is a tetramer of (pol III polypeptide)$_2$ (copol III* polypeptide)$_2$ with a molecular weight of 334,000.

Structure of Pol III and Copol III**—Pol III* has been shown to consist of 90,000-dalton subunits and to co-chromatograph on gel filtration with β-galactosidase (3). The unexpected breadth of the pol III* gel filtration profile may reflect the presence of oligomers ranging from two to six subunits or more. Glycerol gradient sedimentation (Fig. 5) gave a sedimentation coefficient of 7.25 S for pol III*. These data would give quite different molecular weights for a spherical protein; they are consistent with an asymmetric molecule of 360,000, indicating that pol III* is a tetramer of 90,000-dalton polypeptides. Copol III* has a molecular weight of approximately 77,000 (Fig. 6) and is composed of a single 77,000-dalton polypeptide (Fig. 7).

Conversion of Pol III to Pol III*—Pol III* can be converted to pol III by a mild heat treatment, as judged by selective loss of the capacity to replicate templates with long single-stranded regions (Fig. 8) and by shift of its gel filtration profile to that of pol III (data not shown). Data from gel filtration and glycerol gradient sedimentation (1, 3) indicate a molecular weight for pol III consistent with a dimer of the 90,000-dalton *dna*E polypeptide.

Catalytic Properties of Pol III Holoenzyme

Holoenzyme Requires ATP—Our earlier studies of pol III* had shown an ATP requirement for this enzyme when assayed with copol III* on primer templates with long, single-stranded regions (4). This ATP requirement was specific for the formation of a pol III* initiation complex, an event which preceded DNA chain elongation. Pol III*, copol III*, a DNA-binding agent such as spermidine or DNA-unwinding protein, ATP, and primer template were all found necessary for formation of pol III* initiation complex. During this reaction, ATP was cleaved to ADP and Pi, which remained bound to the complex. Holoenzyme has also been found to be dependent on ATP for DNA synthesis on primer templates with long, single-stranded regions (Table III), but, like pol III* and pol III (1, 4), it does not require ATP when filling short gaps (data not shown).

Copol III Functions in Holoenzyme*—Unlike pol III*, holoenzyme does not require additional copol III* for synthesis on templates with long gaps (Table IV); this activity of the holoenzyme is, however, sensitive to antibody against copol III* (Table V), as might be expected from the presence of copol III* polypeptides in the holoenzyme molecule (Figs. 2 and 7). Neither holoenzyme nor pol III* is sensitive to anti-copol III* when assayed on templates with short gaps.

Stimulation of Holoenzyme by Phospholipids—Phospholipid is necessary for optimal holoenzyme activity (Fig. 9). Crude *E. coli* phospholipids or purified *E. coli* phosphatidyl ethanolamine or ox brain diphosphatidylglycerol were equally effective (half-maximal synthesis at 0.15 mg per ml of phospholipid) in supporting DNA synthesis by holoenzyme. They were replaced poorly by bovine serum albumin (25% optimal rate) or by other proteins (<10% optimal rate). Further studies will be necessary to determine the role of phospholipids in this reaction.

Other kinetic parameters of holoenzyme were determined (Fig. 10). Despite its lability and complex structure, holoenzyme can synthesize DNA at a linear rate for at least 5 min (Fig. 10*A*) and this synthesis is proportional to added enzyme (Fig. 10*B*). Like pol III, its activity is optimal at a very low salt concentration

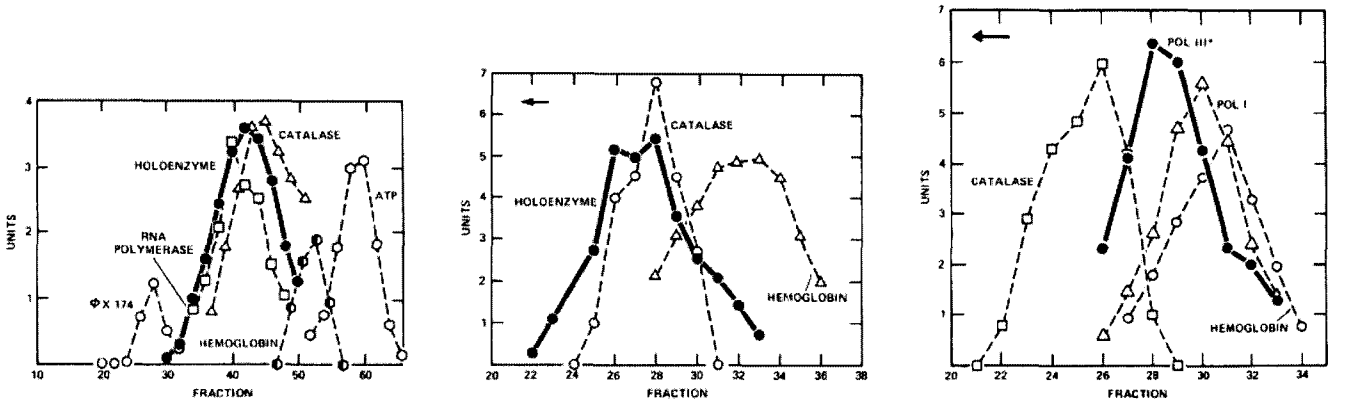

FIG. 3 (*left*). Gel filtration of holoenzyme. Pol III holoenzyme (50 units), ^{3}H-leucine-labeled ϕX174 phage (3,000 cpm), RNA polymerase holoenzyme (0.05 mg), catalase (2 mg), ^{3}H[ATP] (12,000 cpm), and human hemoglobin (0.6 mg) were mixed (sample volume 0.3 ml) and applied to a Bio-Gel A-5m column (1 × 12 cm) equilibrated with 30% glycerol, 0.5 mg of bovine serum albumin per ml, 0.05 M Tris·Cl (pH 7.5) 20 mM dithiothreitol, 0.1 M ammonium acetate, and 1 mM EDTA at 4°. Fractions (0.2 ml) were assayed for ^{3}H, RNA polymerase (11), catalase (A_{540}), hemoglobin (A_{430}), and DNA polymerase III holoenzyme (as described under "Materials and Methods"). One unit on the *ordinate scale* has the following equivalents: ϕX174, 1,000 cpm of ^{3}H (○); RNA polymerase, incorporation of 0.25 pmole of acid-insoluble ribonucleotide (□); DNA polymerase III holoenzyme, incorporation of 10 pmoles of acid-insoluble nucleotide (or 0.01 unit as defined under "Materials and Methods") (●); catalase, A_{406} of 0.05 (△); hemoglobin, A_{430} of 0.025 (*shaded hexagons*); and ATP, 1,000 cpm of ^{3}H (*hexagons*).

FIG. 4 (*center*). Glycerol gradient sedimentation of holoenzyme. Holoenzyme (50 units), catalase (0.25 mg), and hemoglobin (0.50 mg) were sedimented in a glycerol gradient (sample volume 0.15 ml, 3.6 ml, 35 to 50% glycerol gradient). Conditions and assay of holoenzyme sedimentation are described under "Materials and Methods." One unit on the *ordinate scale* has the following equivalents: holoenzyme, incorporation of 10 pmoles of acid-insoluble nucleotide or 0.01 unit as defined under "Materials and Methods" (●); hemoglobin, A_{430} of 0.2 (△); and catalase, A_{406} of 0.025 (○).

FIG. 5 (*right*). Glycerol gradient sedimentation of pol III*. Glycerol gradients were as described under "Materials and Methods." Pol III* (17 units), catalase (4.0 mg), and hemoglobin (0.5 mg) in 0.2 ml were layered on one gradient; a second gradient, run in the same rotor, had a sample (0.2 ml) of hemoglobin (0.5 mg) and pol I (6 μg of Fraction 7 (5)). The peak of hemoglobin was at Fraction 31 in each gradient; results from the two gradients are plotted together for simplicity. One unit on the *ordinate scale* has the following equivalents: hemoglobin, A_{430} of 0.2 (○); pol I, incorporation of 20 pmoles of acid-insoluble nucleotide (△); catalase, A_{406} of 0.02 (□); and pol III*, incorporation of 2 pmoles of acid-insoluble nucleotide (●).

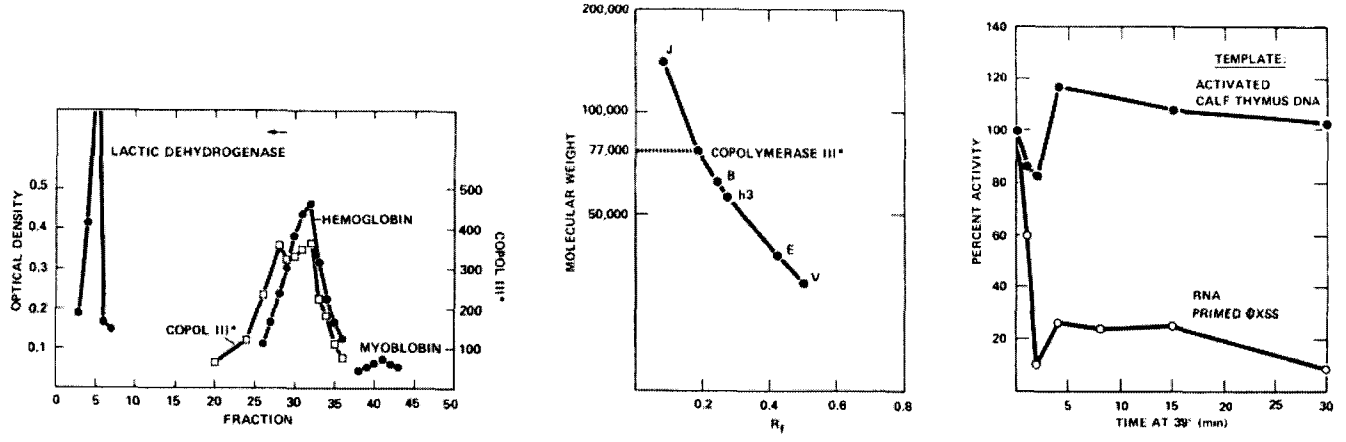

FIG. 6 (*left*). Glycerol gradient sedimentation of copol III*. Copol III* (10 μg), lactic acid dehydrogenase (250 μg), hemoglobin (200 μg), and myoglobin (100 μg) were mixed (in 200 μl) and sedimented through a glycerol gradient (15 to 30% glycerol, 50 mM Tris·Cl (pH 7.5), 20 mM dithiothreitol, and 1 mM EDTA; the gradient was 3.6 ml in a polyallomer tube in an SW 56 rotor. Sedimentation was for 21 hours at 55,000 rpm at 4°. Fifty-one single drop fractions were collected through a 21-gauge needle from the bottom of the gradient and assayed for lactic acid dehydrogenase (A_{280}, Fractions 2 to 8), hemoglobin (A_{430}, Fractions 26 to 36), myoglobin (A_{415}, Fractions 38 to 43), and copol III* (assayed as described (3)).

FIG. 7 (*center*). Sodium dodecyl sulfate gel electrophoresis of copol III*. Copol III* (5 μg) was boiled in 1% sodium dodecyl sulfate-1% β-mercaptoethanol for 1 min and subjected to electrophoresis in a 10% acrylamide-0.25% bisacrylamide slab gel (Beckman Microzone Cell, model R-101) for 3 hours at 50 ma at room temperature. The gel and reservoir buffers of Viñuela *et al.* (12) were used. λ-Head proteins (*J*, *B*, *h3*, *E*, and *V*) were subjected to electrophoresis in parallel as molecular weight standards. We thank Dr. Sherwood Casjens, of this department, for help with this technique.

FIG. 8 (*right*). Heat treatment of pol III*. Pol III* (Fraction V) in 30% glycerol-50 mM Tris·Cl (pH 7.5), 50 mM NaCl, 20 mM dithiothreitol, and 1 mM EDTA (3) was heated at 39° for the times indicated and assayed on activated calf thymus DNA (1) or on RNA-primed ϕX SS DNA (3). Standard activity values (100%) were 10 pmoles of nucleotide incorporated in 10 min at 30° on activated calf thymus DNA and 8 pmoles of nucleotide incorporated in 10 min at 30° on RNA-primed ϕX SS DNA.

TABLE III

Holoenzyme is ATP-dependent

Holoenzyme (0.035 unit) or pol III* (0.044 unit with saturating copol III*) was assayed on $(dT)_9 \cdot (dA)_{1500}$ as described (3), with or without 80 μM ATP.

Form of polymerase	Addition of ATP	DNA synthesis
		pmoles
Pol III* + copol III*	−	0.1
	+	72
Holoenzyme	−	0.2
	+	58

TABLE IV

*Holoenzyme is not stimulated by copol III**

Pol III* (0.006 unit) and holoenzyme (0.006 unit) were assayed on activated calf thymus DNA, on RNA-primed ϕX SS DNA, or on $(dT)_9 \cdot (dA)_{1500}$, as previously described (3). Copol III* (0.1 μg) was added to the assay where indicated.

Form of polymerase	Addition of copol III*	DNA synthesis on templates of		
		Activated calf thymus	RNA-primed ϕX SS DNA	$(dT)_9 \cdot (dA)_{1500}$
		pmoles		
Pol III*	−	87	1	0.1
	+		32	72
Holoenzyme	−	70	28	59
	+		29	69

TABLE V

*Holoenzyme is sensitive to antibody against copol III**

Holoenzyme (0.007 unit) was assayed on RNA-primed ϕX SS DNA as described under "Materials and Methods." Where indicated, holoenzyme was first mixed at 0° with an excess (20 μg) of anti-copol III* (3). Enzyme assays on activated calf thymus DNA were conducted according to the method of Kornberg and Gefter (1).

Template	Addition of anti-copol III*	DNA synthesis
		pmoles
RNA-primed ϕX SS DNA	−	33
RNA-primed ϕX SS DNA	+	4
Activated calf thymus DNA	−	32
Activated calf thymus DNA	+	70

(Fig. 10*C*) and within a broad pH range centered near 7.5 (Fig. 10*D*).

DISCUSSION

Through studies of the replication of single-stranded M13 and ϕX174 viral DNA, we have observed two new forms of DNA polymerase III, physically and functionally distinct from that described originally (1). The new forms have the capacity of pol III to utilize a gapped duplex template but can, in addition, replicate an extensively single-stranded DNA. The form described in this report (holoenzyme) is probably nearest to the form of the polymerase that is functional *in vivo*. It represents all of the pol III identified in a gentle lysate of cells and is a tetrameric protein composed of the two 90,000-dalton polypeptides of polymerase and two 77,000-dalton polypeptides of a copolymerase (Fig. 11). The holoenzyme can be resolved into these components by chromatography on phosphocellulose. The "core" polymerase activity previously isolated as an oligomer (probably a tetramer) of the pol III polypeptides was called polymerase III* (3); the copolymerase essential for pol III* activity on single strands was called copolymerase III*. Our use of the term holoenzyme for the complex of pol III* and copolymerase III* is based on the analogy with the complex of the core polymerase and σ subunit which constitutes RNA polymerase (13). Like the σ subunit, copolymerase III* serves in the initial stage of forming a complex with the template but appears to be dispensable during the replication itself (4).

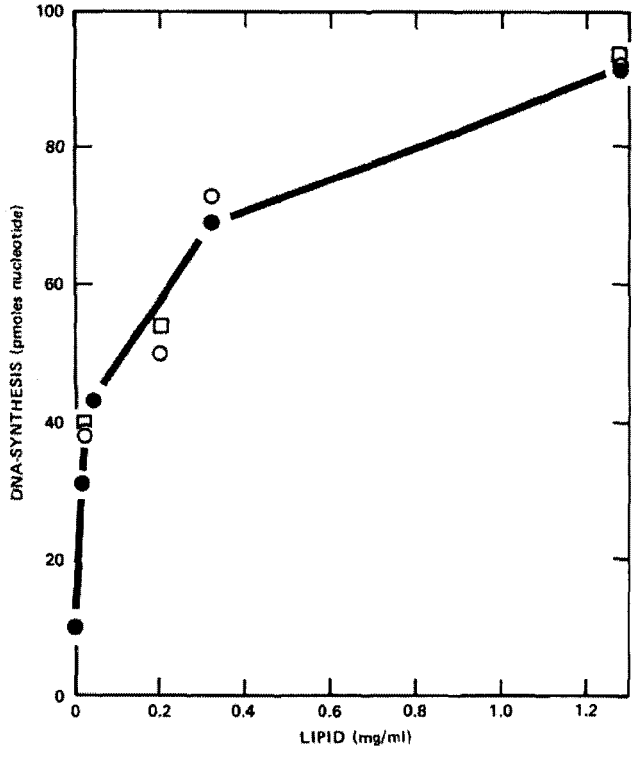

FIG. 9. Phospholipid dependence of holoenzyme activity. Holoenzyme (0.02 unit) was assayed as described under "Materials and Methods," except that the phospholipid concentration was varied. Lipid was total *Escherichia coli* lipid extract (●), *E. coli* phosphatidylethanolamine (○), or ox brain diphosphatidylglycerol (□). The latter two lipids were generous gifts of Dr. E. P. Kennedy, of Harvard University.

Recently, Hurwitz and Wickner (14) have reported the partial purification of a 150,000-dalton protein, termed Factor II, which permits DNA polymerase III to catalyze synthesis on single-stranded templates in the presence of spermidine, DNA-unwinding protein, and copol III*. Unfortunately, their data do not permit an evaluation of whether they used pol III, pol III*, or a mixture of both. These two forms of polymerase could be distinguished by gel filtration (3). Our preparations of pol III* and the holoenzyme have been purified 40,000- and 16,000-fold, respectively, to apparent homogeneity, without our uncovering a requirement for a protein such as Factor II. Conceivably, such a factor could function in the conversion of pol III to pol III*.

The catalytic activities of the several forms of pol III in the replication of the *E. coli* chromosome have not been clarified. In our studies of the structure of these enzymes during catalysis (4), polymerase and copolymerase units form a complex on the primer template during initiation. Still uncertain are the stoichiometry of the proteins and their spatial arrangement in this

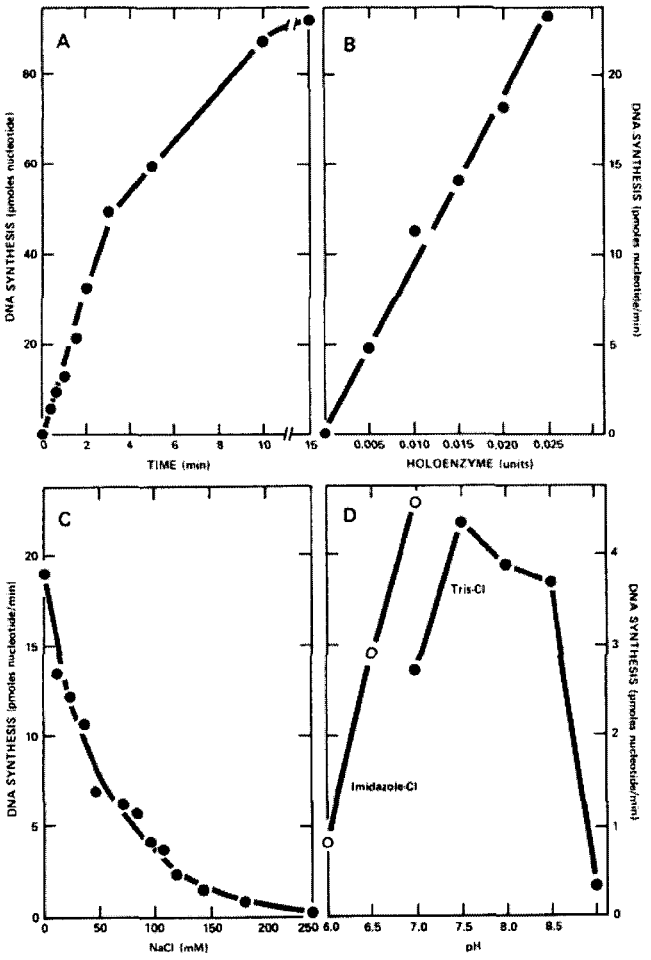

FIG. 10. Properties of holoenzyme. *A*, holoenzyme (0.12 unit) was assayed in a 250-μl reaction mixture as described under "Materials and Methods." Aliquots (25 μl) were withdrawn for determination of acid-insoluble nucleotide. *B*, holoenzyme was assayed for 5 min at 30° in 25-μl reaction mixtures as described under "Materials and Methods." *C*, holoenzyme (0.02 unit) was assayed as described under "Materials and Methods," with additional NaCl as indicated. *D*, holoenzyme (0.005 unit) was assayed as described under "Materials and Methods," but with 10 mM Tris·Cl (pH 7.5) replaced by 50 mM Tris·Cl or imidazole·Cl at the indicated pH.

complex and whether they function as holoenzyme or in some other form of the pol III*-copol III* pair. Even though all of the polymerase III appears as pol III holoenzyme in the gentle lysate, this complex may be associated with additional replication components in the cell.

The functional features of holoenzyme and of pol III* complemented with copol III* are similar, including an absolute dependence on ATP and a sensitivity to anti-copol III*. An important distinction is a dependence of holoenzyme on the presence of phospholipids not consistently observed with pol III*. The lipid factor may provide some structural substitute for the complex in which holoenzyme normally finds itself in the cell.

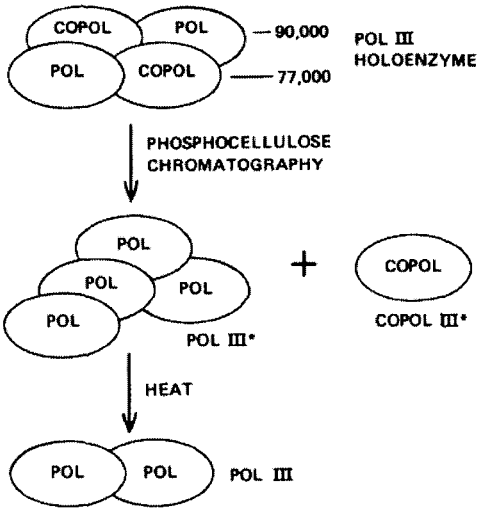

FIG. 11. Hypothetical forms of polymerase III. *Pol* represents the pol III (*dnaE*) polypeptide, and *copol* represents the copol III* polypeptide.

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