THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 252, No. 18, Issue of September 25, pp. 6478-6484, 1977
Printed in U.S.A.

DNA Polymerase III Holoenzyme of *Escherichia coli*

PURIFICATION AND RESOLUTION INTO SUBUNITS*

(Received for publication, April 26, 1977)

CHARLES MCHENRY‡ AND ARTHUR KORNBERG

From the Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

DNA polymerase III holoenzyme has been purified from *Escherichia coli* HMS-83, using, as an assay, the conversion of coliphage G4 single-stranded DNA to the duplex replicative form. The holoenzyme consists of at least four different subunits: α, β, γ, and δ of 140,000, 40,000, 52,000, and 32,000 daltons, respectively. The α subunit is DNA polymerase III, the *dna*E gene product. The holoenzyme has been resolved by phosphocellulose chromatography into an $\alpha \cdot \gamma \cdot \delta$ complex and a subunit β (copolymerase III*); neither possesses detectable activity in the G4 system but together reconstitute holoenzyme-like activity. The $\alpha \cdot \gamma \cdot \delta$ complex has been further resolved to yield a $\gamma \cdot \delta$ complex which reconstitutes $\alpha \cdot \gamma \cdot \delta$ activity when added to DNA polymerase III. The $\gamma \cdot \delta$ complex contains a product of the *dna*Z gene and has been purified from a strain which contains a ColE1-*dna*Z hybrid plasmid.

Conversion of single-stranded circular DNA of phages G4, M13, and ϕX174 to the duplex replicative form requires the synthesis of a primer which is then extended by DNA polymerase III holoenzyme (1). DNA polymerase III, the *dna*E gene product, is inactive in these natural replicative systems; it is able only to fill small gaps in nuclease-treated, duplex DNA (2-4). The holoenzyme,[1] a complex form of pol III, as previously isolated from *Escherichia coli* H560, could be resolved on phosphocellulose to give pol III*, a complex containing the pol III subunit, and copol III* (1). Other investigators studying the conversion of phage SS DNA to RF have isolated several proteins termed DNA elongation factors I, II, and III which are needed for pol III action (5, 6).

In this paper, we report the purification of holoenzyme from *E. coli* HMS-83 and the identification of its subunits. The holoenzyme contains at least four distinct subunits which range in molecular weight from 32,000 to 140,000 (Fig. 1). The DNA polymerase III holoenzyme thus resembles RNA polymerase in complexity but differs in that its subunits are so easily resolved.

* This work was supported in part by grants from the National Institutes of Health and the National Science Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Recipient of postdoctoral fellowship support from a grant from the Cystic Fibrosis Foundation. Present address, Department of Biochemistry and Molecular Biology, University of Texas Medical School at Houston, Houston, Texas 77025.

[1] The abbreviations used are: holoenzyme, DNA polymerase III holoenzyme; pol III, DNA polymerase III; pol III*, DNA polymerase III*; copol III*, copolymerase III*; SS DNA, single-stranded circular DNA; RF, phage double-stranded DNA of circular, replicative form; DBP, DNA binding protein; SDS, sodium dodecyl sulfate.

MATERIALS AND METHODS

Bacterial and Phage Strains—*Escherichia coli* HMS-83 *pol A1 pol B100, thy, lys, lac Z am, rha, str*R (8) was supplied by Dr. Roger McMacken of this laboratory. Strains RLM-365 (pLC 6-2), RLM-365 (pLC 30-3), and RLM-365 (pLC-21), supplied by Dr. Roger McMacken, were constructed by transfer of the corresponding ColE1/*E. coli* hybrid plasmids (9) to RLM-365 (a *pol A*$^+$ revertant of *E. coli* HMS-83). *E. coli* AX-727 *dna* Z$^-$ (10, 11) was obtained from Dr. J. Walker of the University of Texas, Austin. *E. coli* HMS-83 was grown in a broth containing 1% yeast extract, 1% glucose, 1% K_2HPO_4, 0.185% KH_2PO_4, 50 μg/ml of thymine, and 10 μg/ml of thiamin·HCl to an optical density (590 nm) of 7 in a 100-liter Fermacell Fermentor (New Brunswick) at 37° with aeration. The culture was maintained near pH 7.1. Inocula (6 liters) for strain RLM-365 (pLC 6-2) were grown in the presence of a level of colicin E1 which suppresses growth of cells that do not contain a ColE1 plasmid. Cells were harvested rapidly in a Sharples centrifuge (20 to 30 min), suspended in 50 mM Tris·HCl, pH 7.5, 10% sucrose, and rapidly frozen in liquid N_2. Phage G4 (12) was kindly provided by Dr. G. N. Godson of Yale University. Phage G4 DNA was prepared as described (13).

Chromatography Supports—Valyl Sepharose was prepared by a modification of the method of Rimmerman and Hatfield (14). The Sepharose 4B was activated by described procedures (15). Bio-Rex-70 (Bio-Rad Laboratories) is a polyacrylic acid cation exchange resin.

Buffers—These were: I (50 mM imidazole·HCl, pH 6.8, 20% glycerol, 5 mM dithiothreitol, 1 mM EDTA); T (50 mM Tris·HCl, pH 7.5, 20% glycerol, 5 mM dithiothreitol, 1 mM EDTA); Me_2SO (20% dimethylsulfoxide, 10% glycerol, 50 mM imidazole·HCl, pH 6.8, 5 mM dithiothreitol, 1 mM EDTA); 0.20 AS (T + 0.20 g of ammonium sulfate added to each milliliter of buffer); 0.16 AS (same as 0.20 AS except that 0.16 g of ammonium sulfate was used); 2 M AS (I without glycerol and containing 2 M ammonium sulfate); 1.1 M AS (I with 10% glycerol and containing 1.1 M ammonium sulfate); and 0.4 M AS (I containing 0.4 M ammonium sulfate). The pH of all buffer stocks (1 M) was adjusted at room temperature.

Enzymes—Sources were: electrophoretically pure DNA unwinding protein (DBP) (16) and *dna*G protein (Fraction VI, 503,000 units/mg; approximately 40% pure).[2]

DNA polymerase III was partially purified by dialyzing holoenzyme Fraction II (see below; derived from 425 g of *E. coli* HMS-83; 8×10^6 units, 560 mg of protein) overnight against Buffer T (containing 30% glycerol and 25 mM NaCl). This solution was applied to a 110-ml phosphocellulose column equilibrated in Buffer T (containing 30%

[2] S. L. Rowen and A. Kornberg, personal communication.

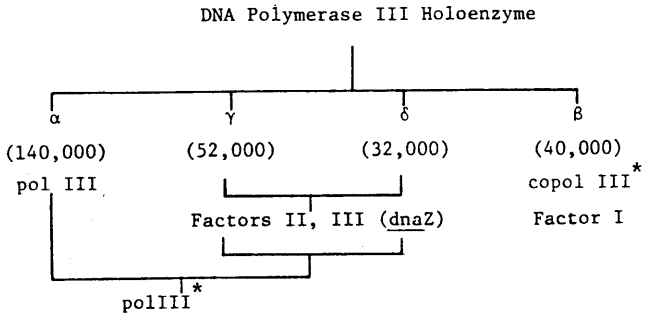

FIG. 1. Components of the DNA polymerase III holoenzyme. This scheme attempts to relate designations made in several laboratories: pol III (2-5), pol III* (1, 3), copol III* (1, 3), Factors I, II, and III (5, 6), *dnaZ* (6, 7).

glycerol and 25 mM NaCl). The activity was eluted by a 6-column volume NaCl gradient (50 → 250 mM) in the equilibration buffer to yield Fraction III (3.3×10^6 units; 14 mg of protein). A portion of Fraction III (one-sixth) was dialyzed for 2 days against 500 ml of Buffer P (20 mM potassium phosphate, pH 6.5, 5 mM dithiothreitol, 25% glycerol) and then applied to a 31-ml phosphocellulose column equilibrated in Buffer P. The column was eluted with a 10-column volume gradient (25 to 200 mM potassium phosphate, pH 6.5, in Buffer P) to yield Frction IV (43 ml, 3.6×10^5 units). Fraction IV was dialyzed overnight in Buffer P and concentrated by adsorption to a 2-ml phosphocellulose column (equilibrated in Buffer P) and elution with Buffer P + 0.175 M potassium phosphate, pH 6.5. The eluted activity was dialyzed against Buffer P overnight to yield Fraction V (1.4 ml, 131 units, 0.12 mg of protein). DNA polymerase III was assayed as described (2). Fraction V was dependent upon both β and $\gamma \cdot \delta$ subunits for activity on an extended single-stranded template.

Assays—DNA polymerase III holoenzyme was assayed routinely using 250 pmol (total nucleotide) of G4 DNA as a template in the presence of saturating quantities of DBP and *dna*G protein (17). Components of holoenzyme were assayed in the presence of saturating amounts of the other subunits. The $\gamma \cdot \delta$ complex was assayed in the presence of α and β; β was added to assays of the $\alpha \cdot \gamma \cdot \delta$ complex and vice versa. Assays in 25 μl were incubated for 20 min at 30°. One unit is defined as 1 pmol of (total) deoxynucleotide incorporated per min; in the preceding paper (1), 1 unit was defined as 1 nmol per min.

In the RNA-primed, ϕX174 assay (as in Table III), 250 pmol of RNA-primed ϕX174 DNA were substituted for G4 DNA. The *dna*G protein,DBP, and rNTPs were omitted. Synthesis was not significantly stimulated by the addition of ATP or DBP. Rifampicin (0.2 μg) was included in each 25-μl assay. Assays were incubated for 5 min. The template was prepared as described (3) except that the ethanol precipitation step was omitted and the product was purified on Bio-Gel A-15m equilibrated in 50 mM Tris·HCl (pH 7.5), 5 mM $MgCl_2$.

Glycerol Gradient Sedimentation—This was performed as described (1) except that the gradients contained 3.7 ml of 25 to 40% glycerol in Buffer I + 0.1 M ammonium sulfate. Sedimentation was for 14 h at 57,000 rpm in a Beckman SW 60 rotor at −5°.

SDS-Polyacrylamide Gel Electrophoresis—Slab gel electrophoresis was conducted in a 10% acrylamide gel (0.275% bisacrylamide) with 0.1% SDS in a Tris·glycine buffer system (18). Protein was labeled with diazotized [^{35}S]sulfanilic acid (19) and bands were detected autoradiographically. Diazotized [^{35}S]sulfanilic acid (2 μl, 1.4 nmol, 4.2 Ci/mmol) was added to 20 μl of protein solution (precipitated with 20% trichloroacetic acid and redissolved in 0.5 M potassium phosphate, pH 7.5), incubated 3 h at room temperature and 15 h at 4°, precipitated with 20% trichloroacetic acid, and dissolved in the SDS-gel buffer. Bands in the holoenzyme preparation detected by this procedure and by Coomassie blue staining were identical.

RESULTS

Purification of Holoenzyme

Holoenzyme was purified 7400-fold to at least 60% homogeneity (Table I). All operations, unless noted, were carried out at 0–4°. Fraction I (10.4 liters) was prepared from 3500 g of *Escherichi coli* HMS-83 as previously described (1).

TABLE I

Purification of DNA polymerase III holoenzyme

Fraction	Total units	Specific activity
	$\times 10^{-3}$	*units/mg protein*
I. Lysate supernatant	20,000	70
II. Ammonium sulfate	14,500	3,900
III. Bio-Rex-70	3,700	16,000
IV. Valyl Sepharose	2,800	90,000
V. DEAE-Sephadex	730	520,000

Ammonium Sulfate Fractionation—Ammonium sulfate (0.226 g/ml) was added over a 10-min interval. The solution was stirred for 30 min at 0° and then centrifuged (23,000 × *g*, 0°, 40 min). The precipitate was resuspended in 0.20 AS buffer (1/8 of the Fraction I volume) and the insoluble fraction was collected. This procedure was repeated with 0.16 AS buffer (1/50 of the Fraction I volume). The dissolved precipitate was clarified by centrifugation (39,000 × *g*, 30 min at 0°) and dialyzed for 4 h against Me_2SO buffer + 0.1 M NaCl to yield Fraction II (156 ml).

Bio-Rex-70 Chromatography.—Fraction II was diluted with Me_2SO buffer (to 312 ml) to a conductivity corresponding to Buffer I + 0.2 M NaCl and applied to a column of Bio-Rex-70 (8.5 cm^2 × 20 cm) equilibrated with Me_2SO buffer. The column was eluted with 8-column volumes of a decreasing Me_2SO gradient (Me_2SO buffer + 0.5 M NaCl → Buffer I + 0.5 M NaCl, 30% glycerol). Me_2SO stabilizes the holoenzyme subunit structure and appears to increase the affinity of proteins for Bio-Rex-70; in its absence, a separate peak of the β subunit was eluted before the holoenzyme. The activity peak (230 ml) was precipitated by dialysis against Buffer I, 70% saturated with ammonium sulfate. The precipitated protein was collected and dissolved in Buffer I + 0.1 M NaCl to yield Fraction III (30 ml).

Valyl Sepharose Chromatography—To Fraction III was added 6 ml of valyl Sepharose (in 1.1 M AS buffer) and 39.6 ml of 2 M AS buffer. The suspension containing protein adsorbed to valyl Sepharose was applied to a valyl Sepharose column (5 cm^2 × 15 cm) equilibrated with 1.1 M AS buffer. The column was washed with 1-column volume of equilibration buffer and eluted with a 10-column volume gradient of 1.1 M AS buffer → 0.4 M AS buffer. Fractions containing at least 50% of the activity in the peak (150 ml) were combined and precipitated by dialysis against Buffer I (70% saturated with ammonium sulfate). The precipitated protein was dissolved in Buffer I to yield Fraction IV (2.35 ml).

DEAE-Sephadex Chromatography—Fraction IV was desalted on a Sephadex G-25 column (1.5 cm^2 × 20 cm) equilibrated in Buffer I + 30 mM NaCl. Fractions preceding the salt peak were immediately applied to a DEAE-Sephadex column (2.2 cm^2 × 9 cm) equilibrated in Buffer I. The column was washed with 2-column volumes of Buffer I + 60 mM NaCl and eluted with 10-column volumes of a NaCl gradient (60 to 210 mM). The activity appeared at about 135 mM NaCl to yield Fraction V (25 ml).

The holoenzyme thus obtained was stable for at least 1 month at 0° and for at least 1 year when rapidly frozen and stored in liquid N_2.

Physical Properties of Holenzyme

Glycerol Gradient Sedimentation—Fraction V (6 ml) was precipitated by dialysis overnight against Buffer I (70% saturated in ammonium sulfate). The resulting fraction was dis-

solved in a minimal volume (100 μl of Buffer I which had been diluted with an equal volume of water), layered upon a glycerol gradient (3.7 ml), and sedimented (see "Materials and Methods"). Of the applied holoenzyme activity, 50% was recovered. The sedimentation profile (Fig. 2) relative to standards in the same rotor indicates a sedimentation coefficient of about 11 S for the holoenzyme, the same value as catalase whose molecular weight is 244,000.

Components of Holoenzyme

SDS-Polyacrylamide Gel Electrophoresis of Holoenzyme—Holoenzyme (0.2 μg) was labeled with diazotized [^{35}S]-sulfanilic acid, denatured, and electrophoresed on an SDS-polyacrylamide gel (Fig. 3). Four protein bands were present which have been shown to be components required for elongation of a primed G4 template (see below). The 140,000-dalton protein (α) is presumably pol III, the *dna*E gene product. Pol III has been purified by others (4) and was found to contain principally a component of this size. A second subunit of 40,000 daltons, β, may be copol III* previously isolated and identified as a 77,000-dalton polypeptide (3). Two components, γ and δ (52,000 and 32,000 daltons, respectively) have been purifed as a complex and are required for the action of α and β (see below). Based upon a densitometer scan of the α-, β-, γ-, and δ-bands, this holoenzyme preparation is at least 60% pure. Also present were two additional proteins of 83,000 and 25,000 daltons, which remained tightly associated with the holoenzyme throughout the purification to this point and sedimented with holoenzyme activity in a glycerol gradient.

Resolution of Holoenzyme—Holoenzyme was resolved by phosphocellulose chromatography into the β subunit and an α·γ·δ complex.[3] The β subunit was further purified on DEAE-Sephadex and Sephadex G-150 (Table II) by a procedure similar to that used for resolving copol III* from a holoenzyme complex (1).

Holoenzyme Fraction V (2 ml) was diluted with 10 mM imidazole·HCl (pH 6.8), 20% glycerol, 5 mM dithiothreitol and 1 mM EDTA (to a conductivity equivalent to Buffer I + 30 mM NaCl) and applied to a phosphocellulose column (0.45 ml) equilibrated with Buffer I + 30 mM NaCl. The column was eluted with 0.5-ml aliquots of Buffer I containing NaCl in the following millimolar concentrations: 30 (twice), 60, 100, 150 (twice), 200, 250, 300, and 400. Two fractions were obtained: an unadsorbed Fraction B (β) (9.4 ml) and an eluted Fraction C (α·γ·δ complex) (1.2 ml; 200 to 250 mM NaCl). Fractions B and C were inactive separately, but together reconstituted holoenzyme-like activity (Table III).

The β subunit was further purified as follows. Fraction B (9.4 ml) was diluted with 2 volumes of Buffer T (1 mM dithiothreitol) and applied to a DEAE-Sephadex column (0.35 ml) equilibrated in Buffer T (1 mM dithiothreitol). The column was washed with 1-column volume of Buffer T and eluted with Buffer T + 0.2 M NaCl. The eluted activity, Fraction D (0.18 ml), was filtered through a Sephadex G-150 column (4 ml, 0.7 cm diameter) equilibrated with Buffer I + 0.1 M NaCl. The eluted activity was frozen and stored in liquid N_2 to yield Fraction E (0.7 ml).

Identification of Subunits of Resolved Holoenzyme Components—Fraction C (0.3 μg) was labeled with diazotized [^{35}S]sulfanilic acid, denatured, and subjected to SDS-polyacrylamide gel analysis. Three distinct protein bands corresponding to the α, γ, and δ subunits were seen (Fig. 3). Additionally, the 83,000- and 25,000-dalton proteins observed in the purified holoenzyme persisted in the α·γ·δ complex. Analysis of Fraction E yielded a single band at 40,000 daltons, corresponding to the β subunit of holoenzyme (Figs. 3 and 4).

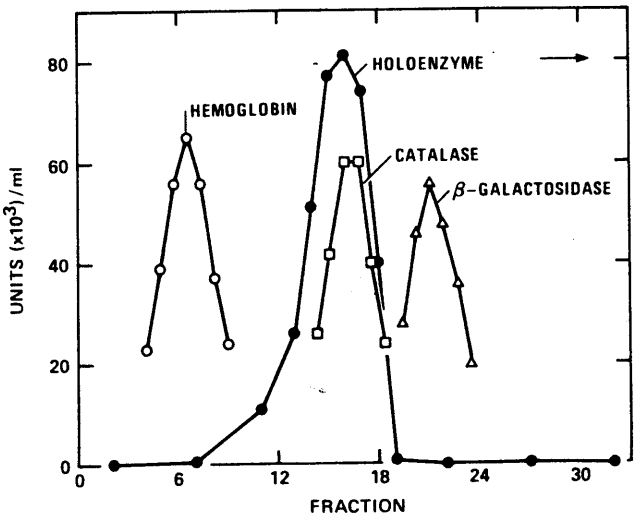

FIG. 2. Glycerol gradient sedimentation of pol III holoenzyme. One unit has the following equivalents: hemoglobin (human), A_{430} of 1×10^{-5}; catalase (beef liver), A_{406} of 5×10^{-6}; β-galactosidase (*Escherichia coli*), A_{420} of 5×10^{-3} (assayed by the method of Craven *et al.* (20)); holoenzyme assayed as described under "Materials and Methods."

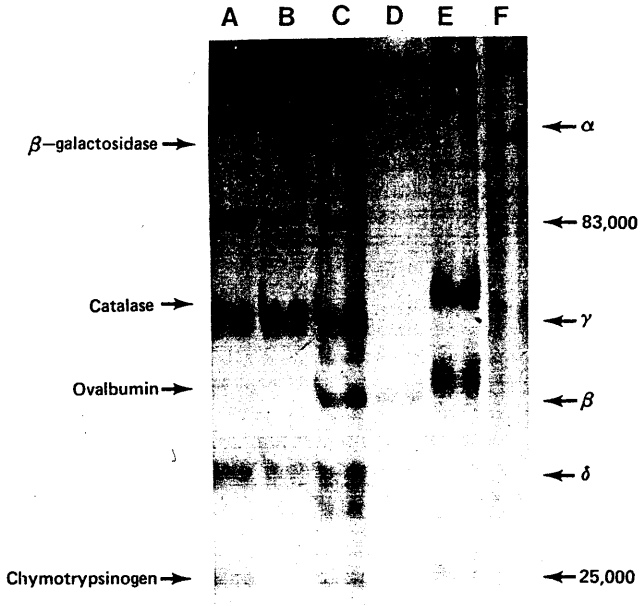

FIG. 3. SDS-gel electrophoresis of holoenzyme and its components. *Lanes A* and *B* contain the designated molecular weight standards. *Lanes C, D, E,* and *F* contain β, holoenzyme, γ·δ complex, and α·γ·δ complex, respectively.

[3] Due to the presumed identity of the α·γ·δ fractions and pol III* and the numerous distinctions between pol III (α) and pol III* (1, 3) it will be tentatively assumed that the α, γ, and δ subunits exist as a complex.

TABLE II

Resolution of holoenzyme into the β subunit and α·γ·δ complex

Fraction	Total units		Specific activity
	α·γ·δ complex	β	
	×10⁻³		units/mg protein
A. Holoenzyme Fraction V	70[a]		620,000
		95[a]	850,000
B. Phosphocellulose, unadsorbed		46	900,000
C. Phosphocellulose, adsorbed	40		700,000
D. DEAE-Sephadex		24	N.D.[b]
E. Sephadex G-150		7	3,600,000

[a] Includes 59,000 units of holoenzyme.
[b] Not determined.

TABLE III

Requirements for reconstitution of holoenzyme activity

Components were added as follows: holoenzyme (14 ng), α·γ·δ complex (15 ng), and β (6 ng); assays are as described under "Materials and Methods."

Holoenzyme component added	DNA synthesis	
	G4 DNA	RNA-primed φX DNA
	pmol	
None	0	0
Holoenzyme	133	20
β	0	0
α·γ·δ complex	8	3
β + α·γ·δ complex	98	19

This band parallels the β activity on a Sephadex G-150 column (Fig. 4). Sedimentation in a glycerol gradient relative to internal standards indicated a sedimentation coefficient of 4.7 S for β (Fig. 5).

Purification of γ·δ Subunit Complex

A complex of the γ and δ subunits of the DNA polymerase III holoenzyme was purified 18,000-fold to 95% homogeneity from *E. coli* RLM 365 (pLC 6-2) (Table IV). Fractions I to IV were prepared as previously described in the purification of holoenzyme except that the assay for the γ·δ complex (see "Materials and Methods") was used to follow activity.

o-Phenanthroline Treatment—The pol III (α) component of the α·γ·δ complex was selectively inactivated and dissociated by treatment with the Zn^{2+} chelator, *o*-phenanthroline, so that the γ·δ complex could be purified as a distinct molecular species. Fraction IV (3.4 ml) was first desalted on a Sephadex G-25 column (1.5 × 17 cm) equilibrated in Buffer T + 100 mM NaCl. To the activity peak was added 0.34 ml of a solution containing 250 mM *o*-phenanthroline·HCl, 200 mM Tris base in Buffer T, and 100 mM NaCl. After 1 h at 0°, the solution was heated for 10 min at 37° to yield Fraction V (4.3 ml).

Phosphocellulose Chromatography—Fraction V was diluted 4-fold with Buffer T and applied to a phosphocellulose column (1 × 14 cm) equilibrated in Buffer T. The column was eluted by a 10-column volume gradient (50 mM to 300 mM NaCl in Buffer T) to yield Fraction VI (37 ml).

DEAE-Sephadex Chromatography—Fraction VI was diluted with Buffer T to a conductivity equivalent to Buffer T + 70 mM NaCl and applied to a DEAE-Sephadex column (0.7 × 7 cm) equilibrated in Buffer T. The column was washed with 1-column volume of Buffer T + 50 mM NaCl and eluted with a

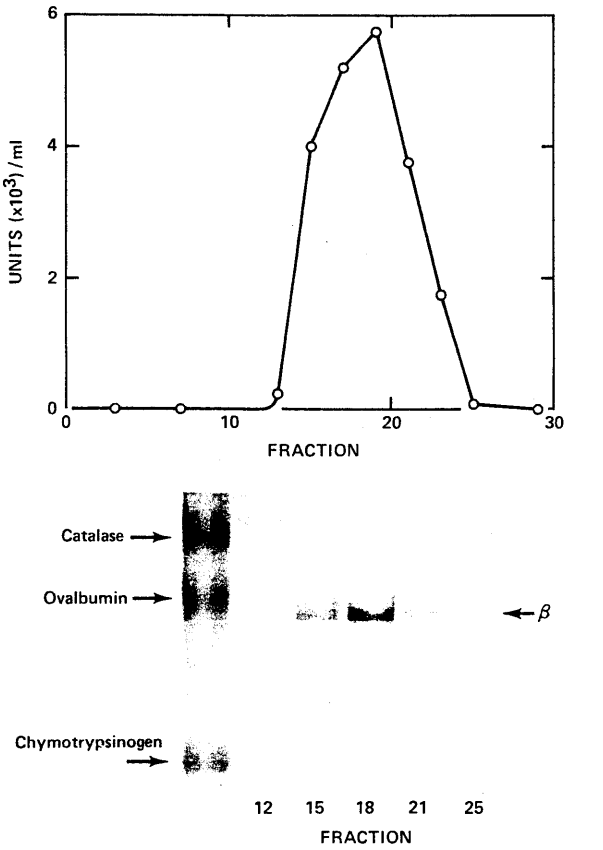

FIG. 4. Chromatography of β subunit of holoenzyme on Sephadex G-150 and electrophoretic analysis of the eluted fractions. β activity was assayed as described under "Materials and Methods"; slab gel electrophoresis of eluted G-150 fractions was in a 10% acrylamide gel with 0.1% SDS. Before electrophoresis, fractions were labeled with diazotized [^{35}S] sulfanilic acid (see "Materials and Methods"). Catalase, ovalbumin, and chymotrypsinogen standards were included in the well on the left side.

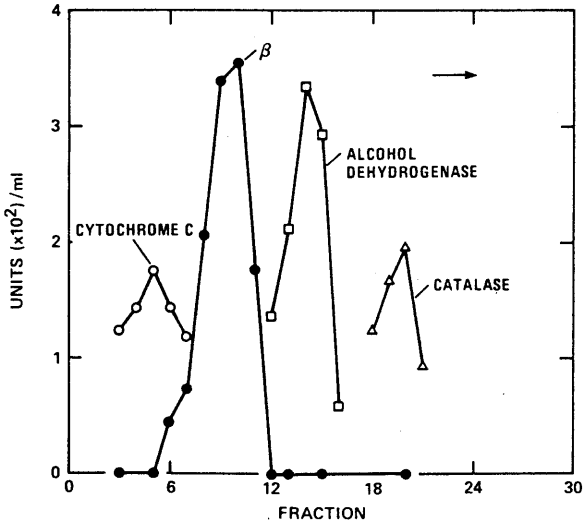

FIG. 5. Glycerol gradient sedimentation of β subunit of holoenzyme. One unit has the following equivalents: cytochrome *c* (horse heart), A_{400} of 2.7 × 10^{-3}; alcohol dehydrogenase (yeast), $\Delta A_{340/min}$ of 3.4 (assayed by the method of Vallee and Hoch (21)); catalase, $\Delta A_{240/min}$ of 3.4 (assayed by the method of Beers and Sizer (22)); and β assayed as described under "Materials and Methods."

10-column volume gradient (75 to 250 mM NaCl in Buffer T). The eluted activity, Fraction VII (6.75 ml), was rapidly frozen and stored in liquid N_2.

Requirement for $\gamma\cdot\delta$ Complex to Reconstitute the Holoenzyme Activity—The $\gamma\cdot\delta$ complex is required to reconstitute holoenzyme-like activity in both the natural G4 assay system and with a long, single-stranded template primed with *E. coli* RNA polymerase (Table V). No activity was observed unless the α, β, and $\gamma\cdot\delta$ subunits were all present.

TABLE IV

Purification of $\gamma\cdot\delta$ subunit complex from a strain carrying a ColE1-dnaZ plasmid

Fraction	Total units		Specific activity
	$\gamma\cdot\delta$	$\alpha\cdot\gamma\cdot\delta$	
	$\times 10^{-3}$		units $\times 10^{-3}$/mg protein
I. Lysate supernatant	203,000	27,000	1.5
II. Ammonium sulfate	140,000	22,000	60
III. Bio-Rex-70	35,000	6,100	320
IV. Valyl Sepharose	26,300	3,400	1,140
V. o-Phenanthroline	12,300	0	540
VI. Phosphocellulose	4,900	0	7,000
VII. DEAE-Sephadex	1,600	0	27,000

TABLE V

Requirement for $\gamma\cdot\delta$ to reconstitute holoenzyme activity

Components were added as follows: holoenzyme (14 ng), β (6 ng), α (55 ng), and $\gamma\cdot\delta$ (1.5 ng); assays are as described under "Materials and Methods."

Holoenzyme component added	DNA synthesis	
	G4 DNA	RNA-primed ϕX DNA
	pmol	
None	0	0
Holoenzyme	184	20
α	0	1
β	0	0
$\gamma\cdot\delta$	0	0
$\alpha+\beta$	0	0
$\alpha+\gamma\cdot\delta$	1	0
$\beta+\gamma\cdot\delta$	0	0
$\alpha+\beta+\gamma\cdot\delta$	142	12

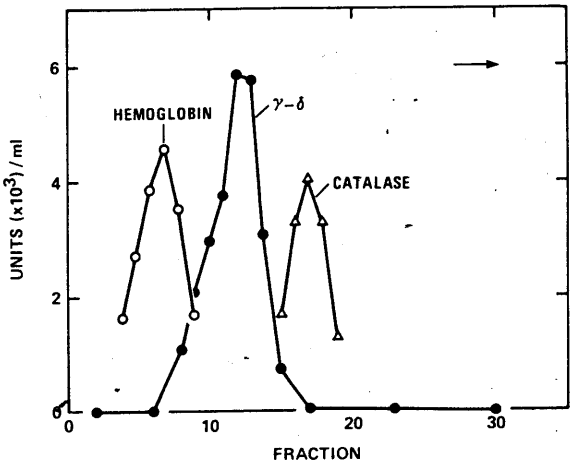

FIG. 6. Glycerol gradient sedimentation of $\gamma\cdot\delta$ subunit complex. One unit has the following equivalents: hemoglobin, A_{430} of 1.5×10^{-4}; catalase, A_{406} of 7.5×10^{-5}; and $\gamma\cdot\delta$ complex assayed as described under "Materials and Methods."

Physical Properties of the $\gamma\cdot\delta$ Complex

Glycerol Gradient Sedimentation—Fraction VII (50 μl) was layered on a 25 to 40% glycerol gradient (3.65 ml) as described under "Materials and Methods," except that NaCl replaced $(NH_4)_2SO_4$. The sedimentation profile (Fig. 6), relative to standards in the same tube, indicates a sedimentation coefficient for the $\gamma\cdot\delta$ complex of 8 S.

SDS-Polyacrylamide Gel Electrophoresis—Fraction VII (0.15 μg) was denatured and electrophoresed on an SDS-polyacrylamide gel (Fig. 3; see "Materials and Methods"). Two distinct protein bands were present with molecular weights of 52,000 and 32.000, respectively. Both of these bands paralleled the activity across a DEAE-Sephadex column (data not shown).

The $\gamma\cdot\delta$ Complex Contains a Product of the dnaZ Gene—When holoenzyme was isolated from a temperature-sensitive *dnaZ* strain, the holoenzyme levels were depressed 10-fold or more (Table VI), as were the levels of the $\alpha\cdot\gamma\cdot\delta$ and $\gamma\cdot\delta$

TABLE VI

Deficiency of $\gamma\cdot\delta$ activity in extracts of a dnaZ mutant

Activity assayed[a]	DNA synthesis	
	HMS-83	AX727 (*dnaZ*)
	units/g cell paste	
Holoenzyme	4500	400
$\alpha\cdot\gamma\cdot\delta$ complex (Fraction C)	4700	600
β (Fraction B)	4400	2700
$\gamma\cdot\delta$ (Fraction VII)	4200	400

[a] Assays of ammonium sulfate functions were performed as described under "Materials and Methods" except that ammonium sulfate precipitation was performed at 0.24 g/ml (instead of 0.226 g/ml as in Table I) and the resulting precipitate was washed with 0.24 AS buffer ($^1/_{10}$ of Fraction I volume).

TABLE VII

Complementation of extracts of a dnaZ mutant by purified $\gamma\cdot\delta$ Complex

Assays were performed as described under "Materials and Methods" in the presence of 2 μg of a Fraction II made from AX727 as described in Table II. The amounts of holoenzyme components added are described in Tables III and V.

	DNA synthesis
	pmol
Fraction II (AX727)	28
$+\alpha\cdot\gamma\cdot\delta$ complex (Fraction C)	137
$+\beta$ (Fraction E)	24
$+\alpha$ (pol III, Fraction V)	28
$+\gamma\cdot\delta$ (Fraction VII)	113

TABLE VIII

Overproduction of $\gamma\cdot\delta$ activity by strains carrying ColE1-dnaZ plasmids

Strain	Plasmid	DNA synthesis	
		Holoenzyme	$\gamma\cdot\delta$
		units/g cell paste	
HMS-83	None	6,000	6,000
RLM 365 (pLC-1-21)	ColE1	8,000	8,000
RLM 365 (pLC-6-2)[a]	ColE1-*dnaZ*	8,000	20,000
RLM 365 (pSC-30-3)	ColE1-*dnaZ*		24,000

[a] In a separate experiment in which the cells were grown under optimal conditions on a large scale (100 liters), 12,000 and 88,000 units/g of cell paste were obtained for holoenzyme and $\gamma\cdot\delta$, respectively.

complexes; β levels were relatively unaffected.

Addition of $\gamma \cdot \delta$ complex to Fraction II of the temperature-sensitive *dnaZ* strain produced a 3-fold stimulation of activity; additions of α or β were without effect (Table VII).

Overproduction of $\gamma \cdot \delta$ Activity in Strains Carrying ColE1-dnaZ Plasmids—Such strains contained levels of $\gamma \cdot \delta$ activity 3 (or more) times greater than strains which lack this plasmid (Table VIII).

DISCUSSION

DNA polymerase III holoenzyme has been purified 7400-fold from *Escherichia coli* HMS-83. The complex is at least 60% pure based upon its content of four subunits: α (140,000 daltons), β (40,000), γ (52,000), and δ (32,000). In addition, proteins of 83,000 and 25,000 daltons, closely associated with the holoenzyme during its purification and subsequent glycerol gradient sedimentation, may be part of the holoenzyme complex serving some unknown functions. The very low abundance of the holoenzyme (20 molecules/cell) and its instability have made its isolation and characterization especially difficult.

The holoenzyme has at least four subunits required for conversion of a primed G4 single-stranded circle to the duplex form. The α subunit is pol III, the *dna*E gene product (23), estimated to be a large polypeptide near 140,000 daltons (2). Others have recently purified pol III and also found it to contain a 140,000-dalton component (4) (Fig. 1). The β subunit of 40,000 daltons is presumably the previously identified copol III* (3) based upon (*a*) association with pol III and stimulation of its activity on a long, single-stranded DNA template, (*b*) resolution from the polymerase component of holoenzyme by phosphocellulose chromatography, (*c*) resistance to treatment with *N*-ethylmaleimide and inhibition by antibody directed against copol III* preparations (data not shown). β and copol III* (3) differ in specific activity and size. The specific activity of β is 3.6×10^6 while that of purified copol III* was 1.7×10^5; their respective sizes measured under denaturing conditions are 40,000 and 77,000 daltons (3). The similarity between copol III* and DNA elongation Factor I noted previously (3, 5) applies to the β subunit as well. The γ and σ subunits of 52,000 and 32,000 daltons, respectively, very likely correspond to the DNA elongation Factors II and III (5, 6). This is based on their similar activities and relationship to the *dna*Z gene. Subunits γ and δ (just as Factors II and III) are required by $\alpha + \beta$ (pol III + Factor I) for action on a single-stranded template. Factor II is the product of the *dna*Z gene (6) as is either γ or δ. (Ref. 7; see below). Inasmuch as the denatured subunit molecular weights for Factors II and III have not been reported, a more precise comparison with subunits γ and δ cannot be made as yet.

The holoenzyme preparation reported here has a specific activity of 520,000 units/mg of protein, a value comparable to that of 400,000 obtained from the previously described procedure using *E. coli* H560 as a source. However, the holoenzyme reported here contains a pol III subunit of 140,000 daltons rather than one of 90,000 as reported previously. Additionally, it contains two subunits (γ and δ) not observed previously in holoenzyme. Whether this difference in subunit composition and molecular weight is due to proteolysis, strain differences or some other cause is uncertain. These two holoenzyme forms do exhibit several common properties: (*a*) similar sedimentation in a glycerol gradient; (*b*) utilization of a primed, long, single strand as template, and (*c*) resolution by phosphocellulose chromatography into two components.

Resolution of the holoenzyme by phosphocellulose chromatography generates the $\alpha \cdot \gamma \cdot \delta$ complex and β subunit, each of which is inactive (in the primed single strand assay systems) but together reconstitute holoenzyme-like activity. The $\alpha \cdot \gamma \cdot \delta$ complex thus exhibits an activity similar to the previously isolated pol III* (3) which was thought to be a complex aggregate of pol III subunits.

The $\alpha \cdot \gamma \cdot \delta$ complex has been further resolved into pol III and a $\gamma \cdot \delta$ complex by treatment with *o*-phenanthroline. This zinc chelator selectively inactivates and dissociates the α subunit permitting the $\gamma \cdot \delta$ complex to be purified as a distinct molecular species. Although zinc has not yet been found to be part of pol III, it is required for the activity of several other RNA and DNA polymerases (24–26). The $\gamma \cdot \delta$ complex shows no detected independent enzymatic activity, but together with the α and β subunits reconstitutes holenzyme-like activity.

The $\gamma \cdot \delta$ complex contains a product of the *dna*Z gene. There is (*a*) a deficiency of this activity in extracts of a *dna*Z mutant, (*b*) a specific complementation of extracts of a *dna*Z mutant strain by the purified $\gamma \cdot \delta$ complex (and not by the α or β holoenzyme subunits), and (*c*) an overproduction of $\gamma \cdot \delta$ activity in a strain carrying extra copies of the *dna*Z gene. Overproduction of $\gamma \cdot \delta$ activity by strains carrying the ColE1/*dna*Z hybrid, even though the *dna*Z gene probably codes for only one of these subunits, could be explained in one of the several ways: (*a*) the plasmid contains the structural genes for both subunits. (*b*) there is a coordinate regulation of the synthesis of the two subunits, or (*c*) the non-*dna*Z gene product is normally produced in excess.

There is a large discrepancy between the high specific activity estimated for $\gamma \cdot \delta$ (2.7×10^7) and that for the holoenzyme (5.2×10^5). It could be explained if the bulk of the holoenzyme preparation were inactive or if the catalytic efficiency of a system reconstituted from isolated α, β, and $\gamma \cdot \delta$ subunits were far greater than that of the holoenzyme.

The argument has been made that the DNA polymerase III holoenzyme is not a physical entity and that its activity derives from a mixture of four separate proteins (27). It seems unlikely to us that four proteins related in a common synthetic function were enriched together throughout a 7400-fold purification simply by coincidence. Furthermore, the components of holoenzyme—α, β, and the $\gamma \cdot \delta$ complex—sediment with coefficients of 7 S (4), 4 S, and 8 S, respectively, whereas the intact holoenzyme sediments with a coefficient of 11 S.

Isolation of a homogeneous DNA III polymerase holoenzyme in amounts adequate for structural and functional characterization remains an important objective. This multisubunit enzyme is crucial in replication not only for its DNA synthetic function but is very likely the keystone of a larger assembly unit containing multiple elements for initiation, regulation, and termination of the replicative operation.

Acknowledgment—We thank Mr. W. Crow for his technical assistance.

REFERENCES

1. Wickner, W., and Kornberg, A. (1974) *J. Biol. Chem.* 249, 6244–6249
2. Kornberg, T., and Gefter, M. L. (1972) *J. Biol. Chem.* 247, 5369–5375
3. Wickner, W., Schekman, R., Geider, K., and Kornberg, A. (1973) *Proc. Natl. Acad. Sci. U. S. A.* 70, 1764–1767
4. Livingston, D. M., Hinkle, D. C., and Richardson, C. C. (1975) *J. Biol. Chem.* 250, 461–469

5. Hurwitz, J., and Wickner, S. (1974) *Proc. Natl. Acad. Sci. U. S. A.* **71**, 6–10
6. Wickner, S., and Hurwitz, J. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73**, 1053–1057
7. McHenry, C. S. (1976) *Fed. Proc.* **35**, Abstr. 1840
8. Campbell, J. L., Soll, L., and Richardson, C. C. (1972) *Proc. Natl. Acad. Sci. U. S. A.* **69**, 2090–2094
9. Clarke, L., and Carbon, J. (1976) *Cell* **9**, 91–99
10. Truitt, C. L., and Walker, J. R. (1974) *Biochem. Biophys. Res. Commun.* **61**, 1036–1042
11. Filip, C. C., Allen, J. S., Gustafson, R. A., Allen, R. G., and Walker, J. R. (1974) *J. Bacteriol.* **119**, 443–449
12. Godson, G. N. (1974) *Virology* **58**, 272–289
13. Zechel, K., Bouché, J.-P., and Kornberg, A. (1975) *J. Biol. Chem.* **250**, 4684–4689
14. Rimmerman, R. A., and Hatfield, G. W. (1973) *Science* **182**, 1268–1270
15. March, S., Parikh, I., and Cuatrecasas, P. (1974) *Anal. Biochem.* **60**, 149–152
16. Weiner, J. H., Bertsch, L. L., and Kornberg, A. (1975) *J. Biol. Chem.* **250**, 1972–1980
17. Bouché, J.-P., Zechel, K., and Kornberg, A. (1975) *J. Biol. Chem.* **250**, 5995–6001
18. Jovin, T., Chrambach, A., and Naughton, M. A. (1964) *Anal. Biochem.* **9**, 351–369
19. Berg, H., and Hirsh, D. (1975) *Anal. Biochem.* **66**, 629–631
20. Craven, G. R., Steers, E., Jr., and Anfinsen, C. B. (1965) *J. Biol. Chem.* **240**, 2468–2477
21. Vallee, B. L., and Hoch, F. L. (1955) *Proc. Natl. Acad. Sci. U. S. A.* **41**, 327–338
22. Beers, R. F., Jr., and Sizer, I. W. (1952) *J. Biol. Chem.* **195**, 133–140
23. Gefter, M. L., Hirota, Y., Kornberg, T., Wechsler, J. A., and Barnoux, C. (1971) *Proc. Natl. Acad. Sci. U. S. A.* **68**, 3150–3153
24. Springgate, C. F., Mildvan, A. S., Abramson, R., Engle, J. L., and Loeb, L. A. (1973) *J. Biol. Chem.* **248**, 5987–5993
25. Auld, D., Kawaguchi, H., Livingston, D., and Vallee, B. (1974) *Biochem. Biophys. Res. Commun.* **57**, 967–972
26. Poiesz, B., Battula, N., and Loeb, L. (1974) *Biochem. Biophys. Res. Commun.* **56**, 959–964
27. Wickner, S. (1976) *Proc. Natl. Acad. Sci. U. S. A.* **73**, 3511–3515