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DNA Polymerase III of *Escherichia coli*

PURIFICATION AND IDENTIFICATION OF SUBUNITS*

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DNA polymerase III, the core of the DNA polymerase III holoenzyme, has been purified 28,000-fold to 97% homogeneity from *Escherichia coli* HMS-83. The enzyme contains three subunits: α, ϵ, and θ of 140,000, 25,000, and 10,000 daltons, respectively. The α subunit has been previously shown to be a component of both DNA polymerase III and the more complex DNA polymerase III holoenzyme (Livingston, D. M., Hinkle, D., and Richardson, C. (1975) *J. Biol. Chem.* 250, 461-469; McHenry, C., and Kornberg, A. (1977) *J. Biol. Chem.* 252, 6478-6484). It is demonstrated here that the ϵ and θ subunits are also subunits of the DNA polymerase III holoenzyme. Thus, the DNA polymerase III holoenzyme contains at least six different subunits. Our preparation has both the 3′ → 5′ and 5′ → 3′ exonuclease activities previously assigned to DNA polymerase III (Livingston, D., and Richardson, C. (1975) *J. Biol. Chem.* 250, 470-478).

Escherichia coli contains three DNA polymerases, designated I, II, and III (for review, see Kornberg, 1974). The central role of DNA polymerase III (Kornberg and Gefter, 1972) in chromosomal replication was established through studies of strains carrying temperature-sensitive, conditional lethal mutations in the *dna* E (*pol* C) gene. These strains, which rapidly cease replication at the nonpermissive temperature, contain defective DNA polymerase III (Gefter *et al.*, 1971). Polymerase III has been isolated in several laboratories and has been shown to contain at least a subunit of 140,000 daltons (Otto *et al.*, 1973; Livingston *et al.*, 1975). Both a 3′ → 5′ and 5′ → 3′ exonuclease activity have been demonstrated in polymerase III (Livingston and Richardson, 1975).

Studies using the natural chromosome probes of phages G4, M13, and ϕX174 have led to the isolation of 12 or 14 different proteins required for the replication of the more complex bacterial chromosome (Schekman *et al.*, 1975; McHenry and Kornberg, 1977; Hurwitz and Wickner, 1974; Wickner and Hurwitz, 1974; Wickner and Hurwitz (1976)). Although these three phage systems differ in their mechanism of priming, all require either the DNA polymerase III holoenzyme[1] for their elongation (Wickner and Kornberg, 1974) or DNA polymerase III plus three additional proteins, Factor I, *dna* Z protein, and Factor III (Wickner and Hurwitz, 1976). Factor I, *dna* Z protein, and Factor III may be holoenzyme components (McHenry and Kornberg, 1977). Resolution and reconstitution of the holoenzyme has shown it to contain at least three subunits β, γ, and δ (40,000, 52,000, and 32,000 daltons, respectively) in addition to the DNA polymerase III catalytic core (McHenry and Kornberg, 1977). Although the polymerase III used in these reconstitution experiments was highly purified (3000-fold), it was not homogeneous. In this paper, we report the purification of DNA polymerase III to homogeneity and the identification of two additional subunits of both DNA polymerase III and the DNA polymerase III holoenzyme.

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[1] DNA polymerase III holoenzyme is the term used for the complex of proteins including the core DNA polymerase III and several auxiliary proteins which are required for the replication of phages G4, M13, ϕX174, and presumably, the *E. coli* chromosome. DNA polymerase III is the name used for the core of the holoenzyme. DNA polymerase III will catalyze limited synthesis upon nuclease treated gapped DNA; only holoenzyme exhibits significant activity on long single-stranded templates.

MATERIALS AND METHODS

Bacterial Strains—*E. coli* HMS-83 *pol* A1, *pol* B100, *thy, lys, lac Z am, rha* (Campbell *et al.*, 1972) was supplied by Dr. Robb Moses of the Baylor College of Medicine. The bacteria were grown as described (McHenry and Kornberg, 1977) to an optical density (600 nm) of 5.5 in a 500-liter fermentor at 36°C. Cells were harvested in 1.5 h in a Sharples centrifuge and immediately frozen in liquid N_2.

Chromatography Supports—The phosphocellulose used for the first column was obtained from Whatman; that used for the second was supplied by Schleicher and Schuell. The hydroxylapatite and AcA34 were products of Clarkson and LKB, respectively.

Buffers—These were: PC (50 mM Tris·HCl (pH 7.5), 30% glycerol, 1 mM EDTA, 5 mM dithiothreitol); HA (50 mM imidazole·HCl (pH 6.8), 1 M KCl, 25% glycerol, 5 mM dithiothreitol); and A (20 mM potassium phosphate (pH 6.5), 5 mM dithiothreitol, 1 mM EDTA, 25% glycerol). The pH of all buffer stocks (0.5 M) was adjusted at 25°C. Dithiothreitol was added to buffers immediately before use from a 0.5 M stock.

Activation of Salmon Sperm DNA—Salmon sperm DNA (Sigma type III) was activated by endogenous nucleases as described (Livingston *et al.*, 1975). The activated DNA was precipitated by the addition of 0.1 volume of 3 M sodium acetate (pH 5.5) and 2 volumes of isopropyl alcohol. After overnight storage at −20°C, the mixture was centrifuged (9500 × *g*, 35 min, 0°C). The resulting pellet was redissolved (5 mg/ml) in 50 mM Tris·HCl, pH 7.5.

Oligonucleotides—[^{3}H]d(T)$_{75}$ was prepared by a modification of the method of Chang and Bollum (1971). d(T)$_{12-18}$ (0.2 A_{260} unit, P-L Biochemicals), [^{3}H]dTTP (0.48 μmol, 0.53 mCi/μmol), and 240 units of terminal transferase (Boehringer) were incubated (1 h, 35°C) in 1 mM $CoCl_2$, 200 mM potassium cacodylate (pH 6.8), and 1 mM dithiothreitol (0.48 ml total volume). The product was purified by filtration through a 35-ml Sephadex G-75 column equilibrated in 10 mM potassium cacodylate, pH 6.8. [^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$ was prepared as above (0.3 ml total volume) using 0.62 A_{260} unit of [^{3}H]d(T)$_{75}$, 0.15 μmol of [^{32}P]dCTP (880 cpm/pmol), and 150 units of terminal transferase. The material obtained by gel filtration was precipitated using sodium acetate/isopropyl alcohol (see above). The estimated lengths of the synthesized oligonucleotides are averages based upon the stoichiometries between the incorporated triphosphates and the starting oligomer.

DNA Polymerase III Assay—The assay was a combination of described procedures (Kornberg and Gefter, 1972; Livingston *et al.*,

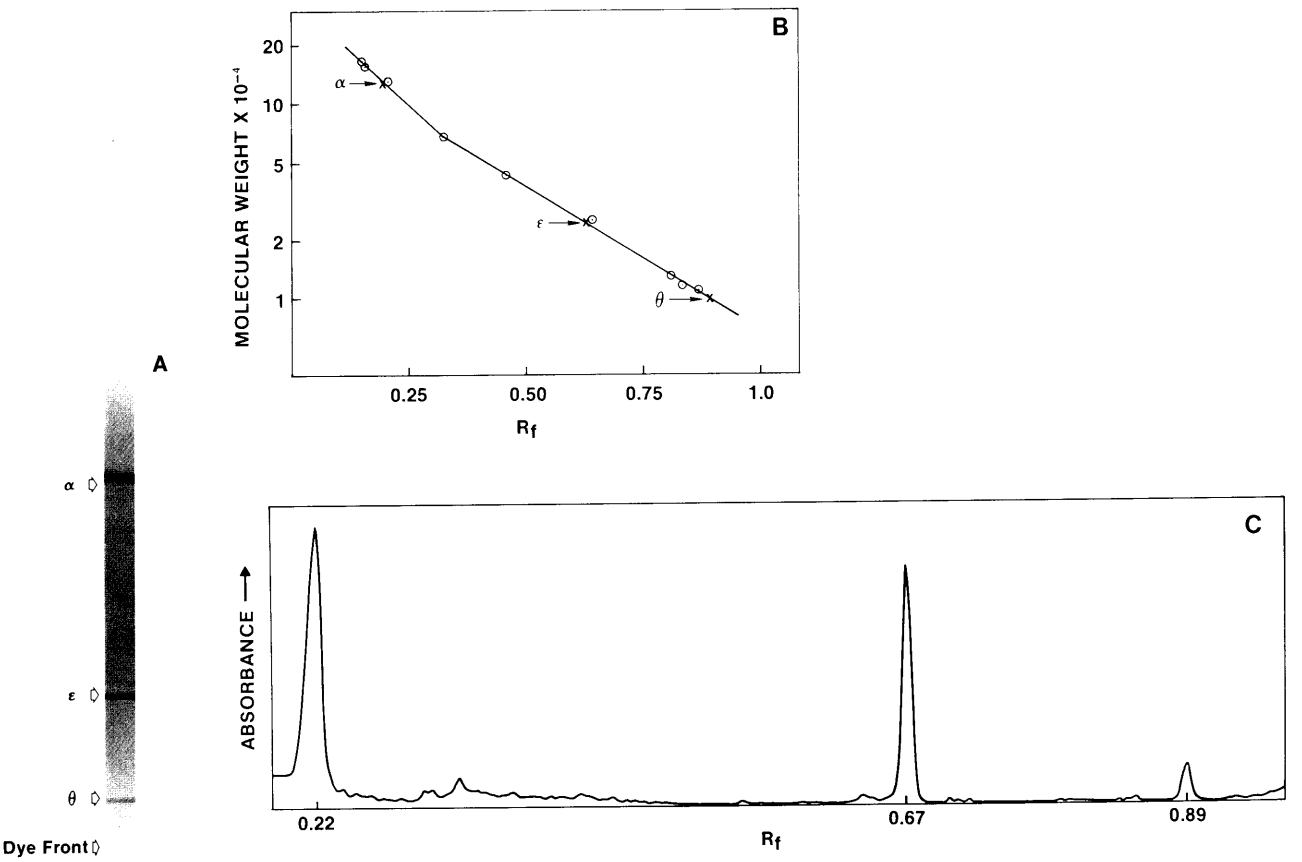

FIG. 1. SDS-gel electrophoresis of DNA polymerase III. *A*, DNA polymerase III (Fraction VI, 6 μg) was denatured, electrophoresed, and stained as described under "Materials and Methods." *B*, determination of denatured molecular weight of DNA polymerase III subunits. Standards of known molecular weight (Weber and Osborn, 1969) (*E. coli* RNA polymerase, *E. coli* β-galactosidase, bovine serum albumin, ovalbumin, chymotrypsinogen, cytochrome *c*, chymotrypsin) were denatured and electrophoresed in adjacent lanes of a slab gel. *C*, a densitometric scan of the gel shown in *A*.

1975). The reaction was initiated by the addition of DNA polymerase III (10 to 40 units) to a solution (30 μl) containing: 33 mM 4-morpholinepropanesulfate (pH 7.0); 17 mM dithiothreitol; 133 μM concentrations of dCTP, dGTP, and dATP; 50 μM [^{3}H]dTTP (approximately 100 cpm/pmol); 13.3 mM $MgCl_2$; 10% ethanol; and 167 μg/ml of activated salmon sperm DNA. If necessary, the enzyme was diluted with 50 mM Tris·HCl (pH 7.5); 20% glycerol; 10 mM dithiothreitol; 0.1 mM EDTA; and 200 μg/ml of bovine serum albumin. After incubation for 5 min at 30°C, the reaction was stopped by chilling and the addition of 2 drops of 0.2 M sodium pyrophosphate and 0.5 ml of 10% trichloroacetic acid. To this was added 2 ml of 1 M HCl, 0.2 M sodium pyrophosphate; the resulting mixture was filtered through a wet Whatman GF/C filter. The filter was washed with four additional aliquots, rinsed with ethanol, and dried, and the radioactivity was determined. One unit is defined as the amount of enzyme catalyzing the incorporation of 1 pmol of deoxynucleotide (total)/min.

SDS[2]-Polyacrylamide Gel Electrophoresis—Slab gel electrophoresis was conducted in a 7.5 to 17.5% acrylamide (0.20 to 0.47% bisacrylamide) gel with 0.1% SDS (Christiansen *et al.*, 1977; Laemmli, 1970). A 5% stacking gel was used. Protein was detected by Coomassie blue staining (0.25% in 45% methanol, 10% acetic acid). Gels were destained in a solution containing 7.5% acetic acid and 10% methanol. Densitometry was performed at 610 nm on a Helena scanning densitometer.

Exonuclease Assay—The reaction was initiated by the addition of DNA polymerase III (6 units) to a solution (30 μl) containing 33 mM 4-morpholinepropanesulfonate (pH 7.0), 17 mM dithiothreitol, 6.7 mM $MgCl_2$, and 100 pmol (total nucleotide) of [^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$. After incubation for 30 min at 39°C, the reaction was stopped by the addition of carrier salmon sperm DNA (70 μl, 0.5 mg/ml) and trichloroacetic acid (100 μl, 10%). The resulting mixture was centrifuged (5 min, 0°C, 20,000 × *g*) and the radioactivity in the supernatant was determined.

[2] The abbreviation used is: SDS, sodium dodecyl sulfate.

TABLE I
Purification of DNA polymerase III

Fraction	Total units × 10^{-4}	Specific activity × 10^{-4}
		units/mg protein
I. Lysate supernatant	14,500	0.11
II. Ammonium sulfate	9,600	4.4
III. Phosphocellulose I	2,900	60
IV. Hydroxylapatite	1,900	190
V. Gel filtration	580	1,500
VI. Phosphocellulose II	210	3,200

RESULTS

Purification of DNA Polymerase III

DNA polymerase III was purified 28,000-fold from *E. coli* HMS-83. All operations, unless noted, were carried out at 0–4°C. Enzyme fractions obtained from chromatographic manipulations which contained at least 50% of the peak activity were combined. Fraction I (5.1 liters) was prepared from 1700 g of cells as described (Wickner and Kornberg, 1974). Fraction II (ammonium sulfate) was prepared as for the DNA polymerase III holoenzyme (McHenry and Kornberg, 1977) except that the resulting pellet was dissolved in Buffer PC + 25 mM NaCl and dialyzed against Buffer PC + 25 mM NaCl overnight to yield Fraction II (88 ml).

Phosphocellulose I—Fraction II (diluted with 84 ml of 10 mM Tris·HCl (pH 7.5), 30% glycerol, 5 mM dithiothreitol to a conductivity equivalent to Buffer PC + 25 mM NaCl) was applied to a phosphocellulose column (46 cm^2 × 7.6 cm)

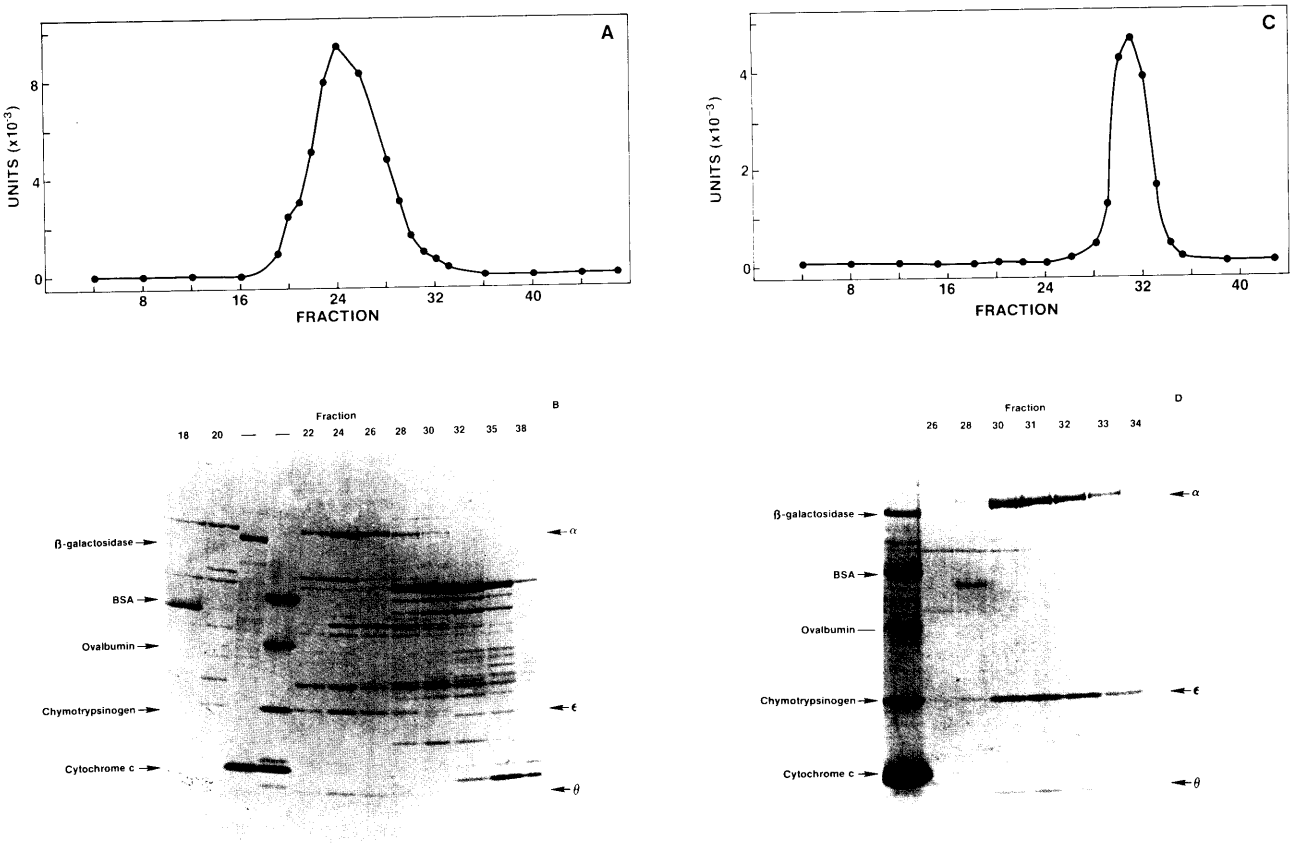

FIG. 2. *A*, gel filtration of DNA polymerase III (Fraction IV) on AcA34 (Step V, see Table I). *B*, SDS-gel electrophoresis of eluted AcA34 fractions. Aliquots (450 μl) of the designated fractions were precipitated with 15% trichloroacetic acid, redissolved in 50 μl of sample buffer, and electrophoresed as described under "Materials and Methods." *C*, chromatography of DNA polymerase III (Fraction V) on phosphocellulose (Step VI, see Table I). *D*, SDS-gel electrophoresis of eluted phosphocellulose fractions. Aliquots (400 μl) treated as described in *B*. *BSA*, bovine serum albumin.

equilibrated with Buffer PC + 25 mM NaCl. The column was washed with 1 column volume of Buffer PC + 50 mM NaCl and the activity was eluted with a 6-column-volume gradient (50 → 250 mM NaCl). Fractions were combined to yield Fraction III (415 ml).

Hydroxylapatite—Fraction III was adjusted to 0.9 M KCl and dialyzed overnight against Buffer HA + 5 mM potassium phosphate. This material was applied to a hydroxylapatite column (2.8 cm^2 × 15 cm) equilibrated with Buffer HA + 5 mM potassium phosphate. The activity was eluted with a 15-column-volume gradient (Buffer HA containing 5 → 100 mM potassium phosphate, pH 6.5). Peak fractions (82 ml) were combined to yield Fraction IV.

AcA34 Gel Filtration—A portion of Fraction IV (50%) was concentrated to 5.5 ml on an Amicon PM-10 membrane (20 p.s.i.) and filtered through an AcA34 column (4.0 cm^2 × 72 cm) equilibrated with Buffer A + 0.1 M KCl (Fraction V, 16 ml).

Phosphocellulose II—A portion of Fraction V (47%, 7.5 ml) was diluted 5-fold with Buffer A (minus EDTA) and applied to a phosphocellulose column (0.8 cm^2 × 1.9 cm). The column was eluted with a 15-column-volume gradient (20 → 200 mM KCl in Buffer A), resulting in Fraction VI (2.5 ml).

The enzyme can be rapidly frozen in liquid N_2 and stored at −60°C where it retains full activity for at least 6 months or it can be adjusted to 50% glycerol and stored at −20°C. Using the latter conditions, the enzyme loses 25% activity in 6 months. A summary of the purification results is shown in Table I.

Components of DNA Polymerase III

SDS-Polyacrylamide Gel Electrophoresis—DNA polymerase III (Fraction VI, 6 μg) was denatured and electrophoresed on an SDS-polyacrylamide gel (Fig. 1*A*). Three protein bands, termed α, ϵ, and θ of 140,000, 25,000, and 10,000 daltons were present (Fig. 1*B*). A densitometric scan (Fig. 1*C*) indicates the ratio of α, ϵ, and θ to be 1:2.5:1.1 after correction of bound dye for molecular weight. Due to the variability of dye binding to different proteins, we will withhold assignment of molar ratios. The 140,000-dalton component has been previously identified as a component of both DNA polymerase III (Otto *et al.*, 1973; Livingston *et al.*, 1975) and the DNA polymerase III holoenzyme (McHenry and Kornberg, 1977).

Co-chromatography of α, ϵ, and θ with Polymerase III Activity—The α, ϵ, and θ proteins co-chromatograph in all purification procedures reported in Table I. It is demonstrated (Fig. 2) that all three proteins increase and decrease with polymerase activity upon gel filtration and phosphocellulose chromatography. We conclude that α, ϵ, and θ are subunits of DNA polymerase III.

Two-dimensional Gel Electrophoresis—To support our conclusion that the α, ϵ, and θ subunits exist as a tight complex, Fraction V was subjected to two-dimensional electrophoresis. The protein was electrophoresed in the native state in one dimension and then denatured and electrophoresed in a second dimension. In the native gel, two bands were obtained (Fig. 3*A*) consistent with the 50% purity of the gel filtration stage material (see Table I). The denaturing second dimension

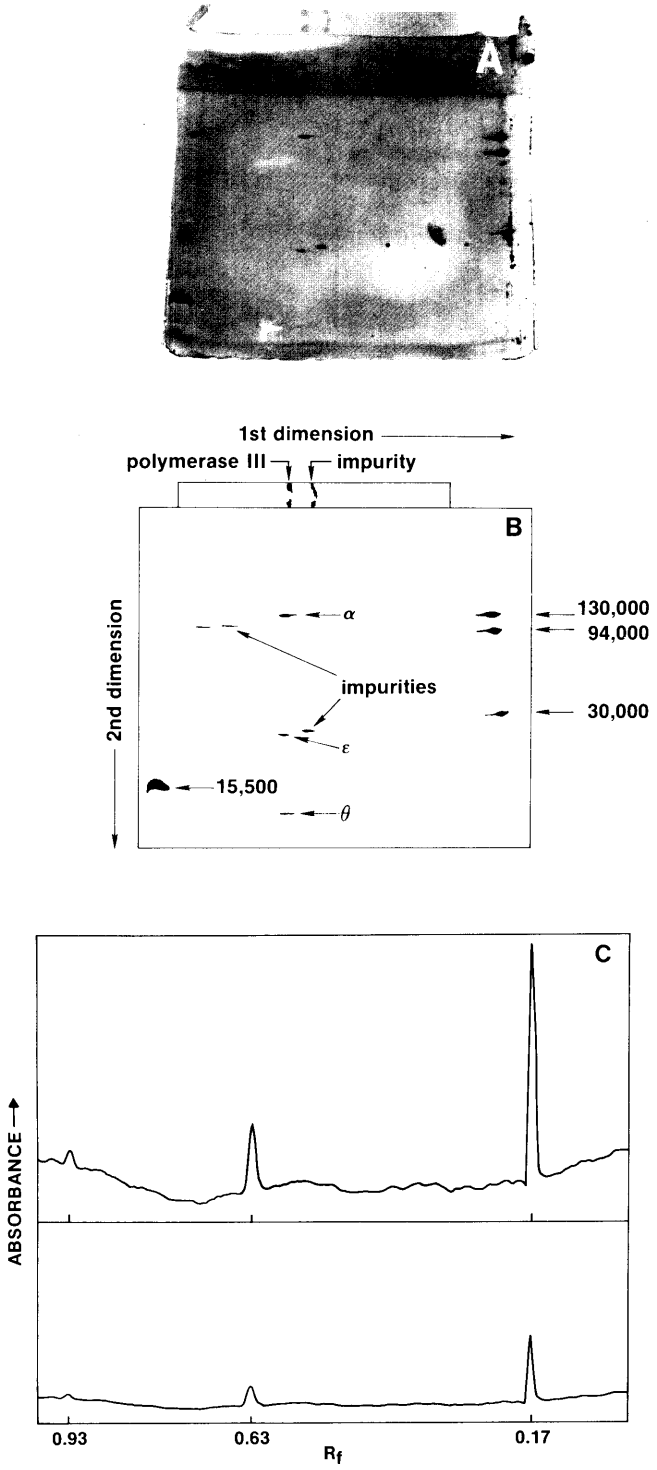

FIG. 3. *A*, two-dimensional electrophoresis of DNA polymerase III. Fraction V (1 ml, 8 μg) was concentrated to 100 μl by vacuum dialysis (collodion bag, 25,000-dalton exclusion). This was applied to a 5% native slab gel which had been pre-electrophoresed overnight against the gel buffer + 2 mg/ml of glutathione. The native gel contained all of the components listed under "Materials and Methods" except SDS. No stacking gel was used. The gel was stained and destained, and the entire lane containing polymerase III was cut out of the slab, photographed, soaked in 0.125 M Tris·HCl (pH 6.8), 1% SDS, 10 mM dithiothreitol, 1% bromphenol blue for 1.5 h with changes in buffer. The gel strip was then sealed in the stacking gel of a standard SDS slab gel and electrophoresed in the second dimension. Standards were included in the side lanes. The entire slab was then stained, destained, and photographed. A photograph of the first dimension is superimposed. *B*, schematic representation of the gel pattern. The molecular weight of the markers run on the side lanes is indicated, as are the identity of the bands of the two-dimensional gel. *C*, a densitometric scan of the lane containing DNA polymerase III. The two scans were performed at different full scale settings.

indicated that one of these two bands contained a complex of the α, ε, and θ components (Fig. 3*A*). The second band (an impurity not present in Fraction VI) contained none of these components. A densitometric scan of the polymerase III containing lane indicates ratios of 1:2:0.9 between α, γ, and δ subunits, respectively (Fig. 3*C*).

Presence of α, ε, and θ in DNA Polymerase III Holoenzyme—The subunits of DNA polymerase III are also present in highly purified DNA polymerase III holoenzyme (Fig. 4). The three DNA polymerase III components co-electrophorese with the corresponding holoenzyme components when run in adjacent lanes of a slab gel (data not shown). The holoenzyme fraction used (Fig. 4) was the most highly purified preparation available; it was purified by a procedure markedly different than that used for the core polymerase III (McHenry and Kornberg, 1977). Based upon this information, ε and θ are assigned as subunits of the DNA polymerase III holoenzyme.

Exonuclease Activity

3′ → 5′ Exonuclease—When the polymer [^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$ was treated with polymerase III, the entire substrate was digested (Table II). Under conditions where polymerase III levels are less than saturating, ^{32}P is preferentially released (Table II). When [^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$ is annealed to a 2.5-fold excess of poly(dA), cytidine is removed without affecting the thymidine (Table II). This effect is not merely due to competition by added nonradioactive polymer since an equivalent amount of oligo(dT)$_{12-18}$ does not inhibit the reaction. Thus, this preparation of polymerase III contains a 3′ → 5′ exonuclease which is specific for single-stranded DNA.

5′ → 3′ Exonuclease—The presence of a 5′ → 3′ exonuclease was established by using the template [^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$ to which excess oligo(dG)$_{12-18}$ was annealed, blocking the 3′ → 5′ exonuclease (see above). Polymerase III removed approximately equal amounts of thymidine and cytidine from this 3′-blocked template (Table III). When the 5′ portion of the substrate is double-stranded, it becomes inert to nuclease attack. This is demonstrated by annealing both poly(dA) and oligo(dG)$_{12-18}$ to [^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$ (Table III). The low level of cytidine hydrolysis is presumably due to a small percentage of nonhybridized 3′ termini. Thus, our DNA polymerase III also contains a 5′ → 3′ exonuclease which must commence hydrolysis on a single strand of DNA but which can proceed into a double-stranded region.

DISCUSSION

DNA polymerase III has been purified 28,000-fold from *E. coli* HMS-83, a *pol* A, *pol* B mutant. The enzyme is at least 97% pure based upon a densitometric scan of the SDS gel shown in Fig. 1. A knowledge of the properties of the multiple forms of DNA polymerase III greatly facilitated this purification. At each stage, the enzyme was maintained in one form to prevent smearing on chromatographic columns, multiple peaks, etc. The enzyme was purified in the holoenzyme form (Wickner and Kornberg, 1974) through the ammonium sulfate step (Fraction II). Phosphocellulose was used to convert holoenzyme to DNA polymerase III* (Wickner *et al.*, 1973). Treatment with 1 M KCl and subsequent hydroxylapatite chromatography permits resolution of the remaining auxiliary holoenzyme components, leaving the core polymerase III. Hydroxylapatite chromatography at lower (0.1 M) salt concen-

FIG. 4. SDS-gel electrophoresis of DNA polymerase III holoenzyme. The subunits common to both holoenzyme and polymerase III are labeled. The holoenzyme used (15 μg) had been sedimented through a glycerol gradient after Fraction V (McHenry and Kornberg, 1977).

TABLE II

3′ → 5′ exonuclease activity

The designated reactions contained 250 pmol of poly(dA) or oligo(dT)$_{12-18}$. All reactions contained 6 units of DNA polymerase III except for that reported in line 4 which contained 0.9 unit.

Substrate	Acid-soluble	
	T	C
	%	%
[^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$	100	100
[^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$·poly(dA)	3	71
[^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$ + oligo(dT)$_{12-18}$	95	97
[^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$ (low level *pol* III)	29	51

TABLE III

5′ → 3′ exonuclease activity

Both reactions contained 1 nmol of oligo(dG)$_{12-18}$. The experiment on line 2 contained 250 pmol of poly(dA).

Substrate	Acid-soluble	
	T	C
	%	%
[^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$·oligo(dG)$_{12-18}$	42	40
[^{3}H]d(T)$_{75}$-[^{32}P]d(C)$_8$·oligo(dG)$_{12-18}$·poly(dA)	0	6

trations results in the preservation of DNA polymerase III*[3]. After the gel filtration step, polymerase III was rechromatographed on phosphocellulose. This was a useful purification step since polymerase III chromatographs differently on phosphocellulose than polymerase III*, the form of the enzyme in the first phosphocellulose step.

The polymerase III obtained from this purification contains three subunits: α, ε, and θ of 140,000, 25,000, and 10,000 daltons, respectively. The molecular weight assignment of the smallest (θ) subunit should be considered tentative due to the lack of reliability of SDS gels for small proteins. All three subunits parallel one another on phosphocellulose and hydroxylapatite chromatography and on gel filtration. For the small subunit to migrate independently with polymerase III on gel filtration, if it were not attached, would require it to be at least a complex of 20 subunits. To support our contention that polymerase III is a complex of the α, ε, and θ subunits, we subjected Fraction V to two-dimensional electrophoresis. In the native dimension, ε and θ co-migrate with α, a known polymerase III component (Otto *et al.*, 1973; Livingston *et al.*, 1975; McHenry and Kornberg, 1977). All impurities present at this penultimate stage of purification migrate elsewhere.

When polymerase III is in the larger holoenzyme form, the ε and θ subunits migrate with the 11 S holoenzyme upon glycerol gradient sedimentation. Thus, the physical properties of ε and θ change with the physical and functional state of polymerase III, of which they are part. Based upon this information and the knowledge that polymerase III comprises the core of the polymerase III holoenzyme (Wickner and Kornberg, 1974; McHenry and Kornberg, 1977), ε and θ are assigned as subunits of the holoenzyme. This brings the total number of different holoenzyme components to six. The M_r = 25,000 protein present in the reported holoenzyme purification (McHenry and Kornberg, 1977) is ε. It was not assigned as a subunit in that paper since the criteria used for a holoenzyme subunit was its ability to reconstitute holoenzyme when added to the core DNA polymerase III. The low molecular weight θ subunit was not detected due to the use of 10% acrylamide gels.

It is noted that the protein pattern for holoenzyme reported in Fig. 3 is more complicated than the illustration in an earlier publication (McHenry and Kornberg, 1977). The regions previously assigned to γ and δ were resolved into several bands by the gradient gels used in the present study. The two bands which migrate between ε and θ were not detected previously for the same reason noted above for θ. The relationship between these additional bands and holoenzyme is under investigation at present.

A discrepancy exists between the subunit structure reported here for polymerase III and that reported by Livingston *et al.* (1975). Using 5% gels for analysis, they reported polymerase III to contain subunits of 140,000 and 40,000 daltons. These authors did note some heterogeneity on native gels and an apparent excess of the 40,000-dalton component (Livingston *et al.*, 1975). Judging from our experience (see above), smaller subunits may have been overlooked. The specific activity of our preparation is approximately 4-fold greater than that reported (Livingston *et al.*, 1975) but part of this difference may be caused by a 2-fold ethanol stimulation (Kornberg and Gefter, 1972) and shorter assay times. Our preparation does not contain a 40,000-dalton component. Considering the number of forms of polymerase III isolated, it may be possible that there was a specific interaction between polymerase III and a 40,000-dalton protein which was not required for any of the enzymatic properties measured (see below). The β subunit of holoenzyme has a molecular weight of 40,000 daltons; however, it dissociates from the polymerase upon phosphocellulose chromatography (McHenry and Kornberg, 1977). As a precaution against proteolysis, we have purified polymerase III in the presence of EDTA, 2 mM phenylmethylsulfonyl fluoride and 0.2 mM tosyl lysyl chloromethyl ketone with equivalent results (data not shown).

We have assayed the highly purified enzyme described in this paper for exonuclease activity and have found our results in agreement with those reported (Livingston and Richardson, 1975). The 3′ → 5′ exonuclease is single strand-specific; the 5′ → 3′ exonuclease must commence digestion upon a single strand but can proceed efficiently into a double-stranded region. Thus, the absence of the 40,000-dalton component does not lead to a loss of either exonuclease activity previously reported.

We have purified polymerase III to facilitate future studies of the DNA polymerase III holoenzyme. DNA polymerase III catalyzes the incorporation of nucleotides into an activated

[3] C. S. McHenry and W. Crow, unpublished observation.

template as efficiently as holoenzyme; therefore, an important question remains as to the function of the auxiliary proteins β, γ, and δ. An investigation of the function of these proteins required for replication of natural chromosomes should provide insight into the elongation process of a true replicative enzyme.

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