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Accessory proteins for DNA polymerase α activity with single-strand DNA templates

[DNA nucleotidyltransferase (DNA-directed)/factors C1 and C2]

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ABSTRACT **Three forms of DNA polymerase α [DNA nucleotidyltransferase (DNA-directed), EC 2.7.7.7] were partially purified from the combined nuclear extract and postmicrosomal supernatant solution of synchronized HeLa cells. These enzymes, designated DNA polymerases α_1, α_2, and α_3, on the basis of their order of elution from DEAE-Bio-Gel, differ in their abilities to utilize single-strand DNA templates. DNA polymerase α_2 has equal catalytic activities with activated and single-strand DNAs as template-primers. DNA polymerase α_1 has only partial catalytic activity with single-strand DNA templates, and DNA polymerase α_3 is essentially inactive with this template. Successive steps of hydrophobic affinity chromatography and phosphocellulose chromatography of DNA polymerase α_2 resolved the polymerase α activity and two protein factors (C1 and C2) that are required for its catalytic activity with a DNA template-primer that contains extended single-strand regions. In the absence of the factors, DNA polymerase α activity is measurable with activated but not single-strand DNA templates. In the presence of the C1 and C2 factors DNA polymerase α activity with single-strand DNA templates is restored to about 75% of the catalytic activity of DNA polymerase α_2 with this template.**

Heterogeneity of DNA polymerase α [DNA nucleotidyltransferase (DNA-directed), EC 2.7.7.7] has commonly been observed during its purification from a variety of cell systems (1–5). This has hampered the establishment of the physical structure of DNA polymerase α and there is, as yet, no general agreement on the physical properties of the core enzyme (6–10). The physiological significance, if any, of this heterogeneity is not clear at this time. Johnston and coworkers (11) showed that mild treatment of a high molecular weight form (200,000–250,000 daltons) of calf thymus DNA polymerase α with 2.5 M urea converts the enzyme to a 155,000-dalton form with the release of a 50,000- to 70,000-dalton protein(s). McKune and Holmes (12) reconstituted the high molecular weight form of polymerase α from the combination of the 155,000-dalton and the 50,000- to 70,000-dalton protein (s). They report that the 50,000- to 70,000-dalton protein(s) enhances the catalytic activity of the polymerase with synthetic polydeoxyribonucleotide templates. Villani *et al.* (9) have recently shown a similar conversion of DNA polymerase α from *Drosophila melanogaster* embryos in the presence of 2.8 M urea (9). The catalytic activity of the 148,000-dalton form of DNA polymerase α from *Drosophila* embryos (9) and a 156,000-dalton form from rat liver (8) were enhanced when associated with four separable proteins of 54,000–64,000 daltons.

We have previously reported the isolation of a protein (C1) from HeLa cells that specifically stimulates the catalytic activity of HeLa DNA polymerase α 20-fold with DNA template-primers that contain extended single-strand regions (13). In this paper we report the isolation and purification of three forms of DNA polymerase α from synchronized HeLa cells, one of which has equal catalytic activity with activated DNA and with DNA template-primers that contain extended single-strand regions. Two proteins (C1 and C2) that are necessary for its catalytic activity with the latter template-primer are resolved from the DNA polymerase α.

MATERIALS AND METHODS

Materials. ^{3}H-Labeled deoxyribonucleoside triphosphates were purchased from New England Nuclear. Unlabeled deoxyribonucleoside triphosphates and poly- and oligodeoxyribonucleotide homopolymers were from P-L Biochemicals. Calf thymus DNA (Sigma, grade I) was further purified by the procedure of Marmur before use (14). Calf thymus DNA activated by pancreatic DNase was prepared as described (15). Single-strand and "loop-back" forms of calf thymus DNA were isolated by hydroxylapatite chromatography at 60°C (16). Native calf thymus DNA was isolated by chromatography on benzoylated, naphthoylated DEAE-cellulose (BND-cellulose) (Boehringer Mannheim) (17). Exonuclease III-treated calf thymus DNA was prepared by the procedure of Kornberg and Gefter (18). Bacteriophage fd [^{3}H]DNA was prepared according to the procedure of Sadowski and Hurwitz (19). Form I [^{3}H]DNA of simian virus 40 (SV40) was a gift from H. Ozer (Hunter College). ^{3}H-Labeled *Escherichia coli* DNA was prepared as described (13). Native and denatured DNA-cellulose were prepared according to the procedure of Alberts and Herrick (20). All other reagents were of reagent or ultra-pure grade and have been described elsewhere (13, 15, 21).

Growth and Synchronization of HeLa Cells. HeLa S_3 cells were grown according to our published procedures (13, 21) in Joklik's modified Eagle's medium supplemented with 3.5% each of irradiated calf and fetal bovine serum (Irvine Scientific, Irvine, CA). Cells were synchronized by the double hydroxyurea block technique and judged free from *Mycoplasma* contamination by our previously described procedure (13, 21).

Subcellular Fractionation Procedure. The separation of the cell homogenates into the nuclear and postmicrosomal supernatant fractions was according to our published procedures (3, 21, 22). In this case, however, the homogenization was performed in STKMD buffer (250 mM sucrose/50 mM Tris·HCl,

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Abbreviations: ammediol, 2-amino-2-methyl-1,3-propane diol; MalNEt, *N*-ethylmaleimide; SV40, simian virus 40; TKDE, Tris/KCl/dithiothreitol/EDTA; TDE, Tris/dithiothreitol/EDTA.

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pH 7.5 at 25°C/25 mM KCl/5 mM $MgCl_2$/1 mM dithiothreitol) and 1 mM aminoacetonitrile.

DNA Polymerase Assay. The assay of DNA polymerase α activity with activated (15) and single-strand DNA template-primer (13) was performed according to our published procedures. The assays with poly(dA)·$(dT)_{12-18}$ as the template-primer were by the procedure of Holmes *et al.* (2). DNA polymerase β activity was assayed with activated DNA as template-primer by our published procedures (21). DNA polymerase γ activity was assayed according to the procedure of Knopf *et al.* (23) with poly(rA)·$(dT)_{12-18}$ as template-primer.

DNase Assays. The assay conditions were identical to those used for the assay of DNA polymerase α except that fd [^{3}H]DNA, *E. coli* [^{3}H]DNA, or SV40 form I [^{3}H]DNA served as the substrate. Exo- and endodeoxyribonuclease activities were determined as described (13).

Purification of DNA Polymerase α. The starting material for the purification was the combined nuclear extract and postmicrosomal supernatant solution (S-3) derived from 25–100 g of synchronized HeLa cells harvested during mid-S phase of the cell cycle. The combined fractions were dialyzed against TKDE (50 mM Tris·HCl, pH 7.5/25 mM KCl/1 mM dithiothreitol/1 mM Na_3EDTA) buffer/10% (vol/vol) glycerol/1 mM aminoacetonitrile and chromatographed on a column of DEAE-cellulose that was equilibrated with TKDE buffer. After loading of the sample, the column was eluted with 8 column volumes of TDE (50 mM Tris·HCl, pH 7.5/1 mM dithiothreitol/1 mM Na_3EDTA) buffer/0.3 M KCl/10% glycerol. Over 90% of the DNA polymerase activity was recovered in the combined column flow-through and 0.3 M KCl eluate. This fraction was dialyzed against TDE buffer/0.1 M KCl/10% glycerol and loaded onto a column of native DNA-cellulose that was coupled to a column of denatured DNA-cellulose. The DNA-cellulose columns were equilibrated with TDE buffer/0.1 M KCl/10% glycerol. After loading, the native DNA-cellulose column was washed with 10 column volumes of TDE buffer/0.1 M KCl/10% glycerol and the effluent was loaded directly onto the denatured DNA-cellulose. The column flow-through fraction from denatured DNA-cellulose contained DNA polymerase α activity as determined from its inhibition by 2.5 μM aphidicolin (24), 100 μM butylanalinouracil (25), or 1 mM *N*-ethylmaleimide (MalNEt) and its insensitivity to 2′,3′-dideoxythymidine 5′-triphosphate (26, 27). This fraction was dialyzed against TKDE buffer/10% glycerol and chromatographed on a DEAE-Bio-Gel (Bio-Rad) column equilibrated with the dialysis buffer. The column was washed with 8 column volumes of TKDE buffer/10% glycerol and then eluted by a discontinuous gradient of increasing KCl concentration in TDE buffer/10% glycerol. DNA polymerase α activity was recovered in the column flow-through fraction, the 0.1 M KCl, and the 0.3 M KCl eluates.

Polyacrylamide Gel Electrophoresis Under Nondenaturing Conditions. This electrophoresis was carried out essentially according to the procedure of Holmes *et al.* (11), using 5% nondenaturing cylindrical gels. Samples for electrophoresis were dialyzed against the loading buffer [8 mM 2-amino-2-methyl-1,3-propanediol (ammediol)/30 mM glycine, pH 9.0/2 mM dithiothreitol/20% (vol/vol) glycerol] before electrophoresis.

$NaDodSO_4$/Polyacrylamide Gel Electrophoresis. Gels were prepared and run as described by Laemmli (28). Gels were prepared at acrylamide concentrations of 5%, 7%, 8%, or 10% and were overlayered with a 3% acrylamide stacking gel.

Other Methods. Protein concentrations were measured by the method of Schaffner and Weissman (29), using bovine serum albumin as the standard, or by the absorbance at 280 nm in the case of purified chromatographic fractions.

RESULTS

DEAE-Bio-Gel chromatography at pH 7.5 of the DNA polymerase α activity in the flow-through fraction from denatured DNA-cellulose yielded three chromatographic fractions of DNA polymerase α (Fig. 1). About 30% of the activity did not bind to DEAE-Bio-Gel and was recovered in the column flow-through fraction. Approximately 60% of the DNA polymerase activity was eluted by 0.1 M KCl and 5–10% was eluted by 0.3 M KCl. As in the case of the α-class DNA polymerase, the DNA polymerase activity in each of these chromatographic fractions was inhibited over 95% by 2.5 μM aphidicolin or 1 mM MalNEt. The DNA polymerase activities in the column flow-through fraction and the 0.1 M KCl and 0.3 M KCl eluates are designated DNA polymerase α_1, α_2, and α_3, respectively. DNA polymerase α_2 has equal activities with denatured and activated DNAs as template-primers. However, DNA polymerase α_1 is only about one-half as active with denatured DNA as with activated DNA, and DNA polymerase α_3 is essentially inactive with denatured DNA as template-primer.

Rechromatography on DNA-Cellulose and DEAE-Bio-Gel. DNA polymerase α_2 activity was again recovered in the column flow-through of denatured DNA-cellulose and the 0.1 M KCl eluate of DEAE-Bio-Gel during successive steps of rechromatography (data not shown). It retained equal catalytic activity with activated and denatured DNA template-primers after these steps of rechromatography. DNA polymerase α_2 was also eluted by 0.1 M KCl from DEAE-Bio-Gel after gel filtration on Bio-Gel A-1.5m in the presence of 0.5 M KCl (data not shown). Its activities with activated and denatured DNA template-primers were equal and coincided during gel filtration and rechromatography on DEAE-Bio-Gel.

DNA polymerases α_1 and α_3 also did not bind to denatured DNA cellulose upon rechromatography. During subsequent rechromatography of polymerase α_1 on DEAE-Bio-Gel about 60% of the activity was recovered in the column flow-through fraction and 20% was recovered in the 0.1 M KCl eluate. DNA polymerase α_3 activity was recovered in the 0.3 M KCl eluate during subsequent rechromatography on DEAE-Bio-Gel. Their

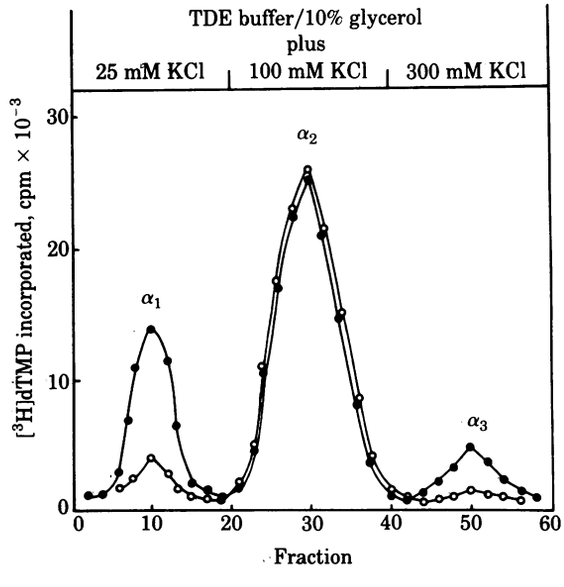

FIG. 1. Resolution of three forms of DNA polymerase α by chromatography on DEAE-Bio-Gel. The column flow-through fraction from denatured DNA-cellulose was chromatographed on a 1 × 10 cm column of DEAE-Bio-Gel and the fractions were pooled, dialyzed, concentrated, and assayed with activated (●) and heat-denatured (○) DNA templates.

catalytic activities with activated and denatured DNAs as template-primers were unchanged.

Polyacrylamide Gel Electrophoresis Under Nondenaturing Conditions. When the purified DNA polymerase α_2 was analyzed by electrophoresis in a 5% nondenaturing gel, a single protein band was observed after staining with Coomassie blue (Fig. 2). The DNA polymerase activity assayed in slices from replicate gels with activated and denatured DNA template-primers had an R_M equal to that of the protein band. Moreover, the activities with activated and denatured DNA template-primers coincided. The molecular weight of DNA polymerase α_2 calculated from electrophoresis in nondenaturing polyacrylamide gels is about 600,000. At this stage of purification DNA polymerase α_2 activity has been purified about 260,000-fold over the activity measured in the combined nuclear extract and S-3 fraction.

Electrophoresis of DNA polymerase α_3 on 5% nondenaturing polyacrylamide gels also gives a single Coomassie blue staining band (unpublished data). DNA polymerase activity assayed in replicate gels with activated DNA as template-primer had the same R_M as the protein staining band. The molecular weight of DNA polymerase α_3 from electrophoresis under nondenaturing conditions is calculated as 220,000. Its activity at this stage in the purification has been purified 200,000-fold over the activity measured in the combined nuclear extract and S-3 fraction.

DNA polymerase α_1 is impure and yields at least eight Coomassie blue staining bands in the molecular weight range of 35,000 to 320,000 after electrophoresis on 5% nondenaturing polyacrylamide gels. The DNA polymerase activity in duplicate gels measured with activated DNA as template-primer coincided with the Coomassie blue stained band for the 320,000-dalton protein.

Hexylagarose Chromatography. DNA polymerases α_2 and α_3 eluted at different positions during hydrophobic affinity chromatography on hexylagarose (Fig. 3). The α_3 polymerase did not bind to hexylagarose, and over 80% of the activity was recovered in the column flow-through fraction. Its activity with denatured DNA as template-primer remained negligible. DNA polymerase α_2 bound to hexylagarose and was eluted by 0.5% Triton X-100. About 80% of the activity with activated DNA but less than 30% of its activity with denatured DNA as template-primer was in this eluate. Additional DNA polymerase activity with activated or denatured DNA as template-primer was not recovered by further elution of the column under a variety of conditions. However, recombination studies with dialyzed chromatographic fractions showed that the 1% Triton X-100

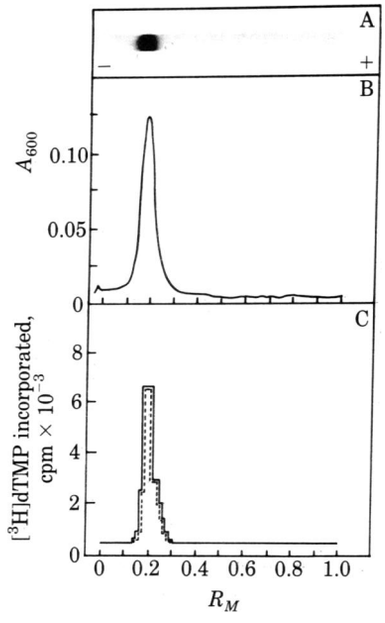

FIG. 2. Nondenaturing polyacrylamide gel electrophoresis of DNA polymerase α_2. The 0.1 M KCl eluate (polymerase α_2) from DEAE-Bio-Gel chromatography was rechromatographed on denatured DNA-cellulose and DEAE-Bio-Gel. The 0.1 M KCl eluate from the second DEAE-Bio-Gel chromatographic step was dialyzed against the loading buffer (8 mM ammediol/30 mM glycine, pH 9.0/ 2 mM dithiothreitol/ 20% glycerol) for nondenaturing gel electrophoresis. The dialyzed samples were loaded onto a 5% nondenaturing gel (0.6 × 9 cm) prepared in 40 mM ammediol/150 mM glycine, pH 9.0/5% acrylamide/0.15% bisacrylamide/0.5 μl of *N,N,N',N'*-tetramethylethylenediamine per ml/0.25% ammonium persulfate/10% glycerol. After polymerization the gels were pre-electrophoresed overnight at 4°C and 4 mA per gel, using 40 mM ammediol/150 mM glycine, pH 9.0/5 mM thioglycollic acid/10% glycerol as the electrode buffer. This was replaced with fresh cold buffer. The dialyzed enzyme (2.5 μg, 50 units) was applied to three gels. Electrophoresis was performed at 4°C and a constant current of 4 mA per gel for 2–3 hr. (*A*) One gel was stained with Coomassie blue. (*B*) Tracing of the gel in *A* in a Helena densitometric scanner. (*C*) Two gels were sliced into 2-mm slices and the slices were extracted. Aliquots of each gel were assayed for DNA polymerase activity with activated (——) or heat-denatured (-----) DNA as template. R_M, relative migration.

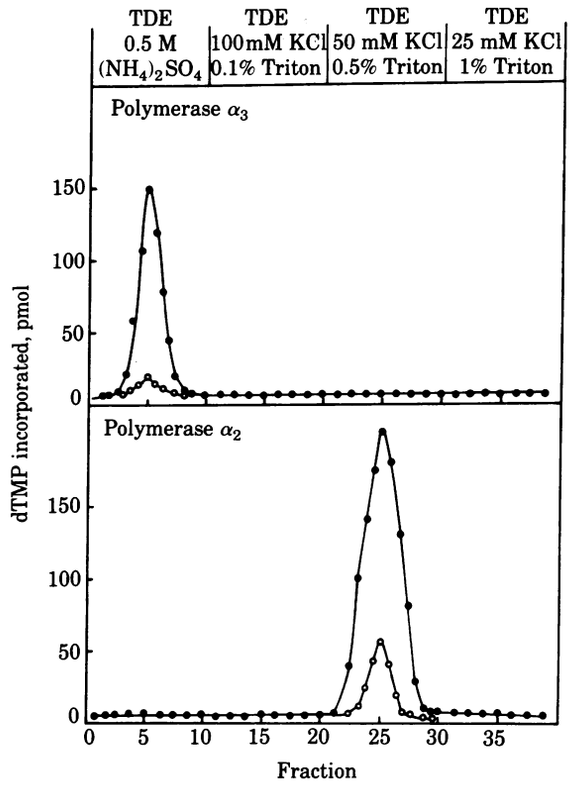

FIG. 3. Hydrophobic affinity chromatography of DNA polymerase α_2 and α_3. DNA polymerases α_2 and α_3 from DEAE-Bio-Gel chromatography were dialyzed against TDE buffer overnight and concentrated by dialysis against solid sucrose. $(NH_4)_2SO_4$ was added to 0.5 M and after 1 hr at 4°C the samples were loaded onto 1 × 2 cm columns of hexylagarose that were equilibrated against TDE buffer/0.5 M $(NH_4)_2SO_4$. The columns were washed with 8 column volumes of the column equilibration buffer and then eluted by a discontinuous gradient of increasing Triton X-100 concentration from 0.1% to 1% and decreasing KCl concentration from 100 to 25 mM in TDE buffer. The eluted fractions were dialyzed against 20 mM potassium phosphate, pH 6.8/1 mM dithiothreitol/1 mM EDTA/10% glycerol and concentrated against solid sucrose. Aliquots (10 μl) were assayed for DNA polymerase activity with activated (●) or heat-denatured (○) DNA as templates.

eluate stimulated the polymerase activity in the 0.5% Triton eluate about 3-fold with denatured DNA as template-primer.

Phosphocellulose Chromatography. The DNA polymerase (0.5% Triton eluate) and stimulatory activity (1% Triton eluate) from hexylagarose chromatography were chromatographed separately on phosphocellulose. The DNA polymerase bound to phosphocellulose at pH 6.8 and was eluted by 0.25 M potassium phosphate. Eighty-five to 90% of the polymerase activity with activated DNA as template was recovered in this eluate. It had essentially no catalytic activity with denatured DNA template-primer. The phosphocellulose step also resolved a second stimulatory factor from the DNA polymerase α activity. The stimulatory factor bound tightly to phosphocellulose and was eluted by 0.5 M potassium phosphate. We designate this stimulatory activity as the C2 factor.

The stimulatory activity that was eluted from hexylagarose by 1% Triton also bound tightly to phosphocellulose and was eluted by 0.5 M potassium phosphate. We designate this stimulatory activity as the C1 factor.

Physical Properties of the Purified DNA Polymerase α, C1, and C2 Proteins. The physical properties of the purified DNA polymerase α activity and C1 and C2 proteins that are resolved from DNA polymerase α_2 by successive steps of chromatography on hexylagarose and phosphocellulose are summarized in Table 1. DNA polymerase α is an acidic protein and appears to be composed of a single polypeptide of approximately 140,000 daltons. The C1 protein is a basic protein of approximately 96,000 daltons and appears to be a tetramer of four identical polypeptide chains of 24,000 daltons. The C2 protein is also a basic protein; it is approximately 51,000 daltons and is composed of a single polypeptide chain.

C1 and C2 Factors Are Required for Synthesis with Single-Strand DNA Templates. DNA polymerase α_2 that is resolved by DEAE-Bio-Gel chromatography (Fig. 1) effectively utilizes polydeoxyribonucleotide templates that contain extensive single-strand regions (Table 2). The template may be either a natural or a synthetic polydeoxyribonucleotide, and the required 3′-hydroxyl primer may be provided by oligodeoxyribonucleotides or oligoribonucleotides or by loop-back regions at the 3′-hydroxyl terminus of single-strand DNA. In the latter case, alkaline sucrose gradient analysis of the reaction product showed a covalent linkage of the template and synthesized product (data not shown).

Table 1. Physical properties of the purified DNA polymerase α and the C1 and C2 proteins

Property	DNA polymerase α	C1 protein	C2 protein
Molecular weight in native state	140,000	96,000	51,000
Molecular weight of subunit	138,000	24,500	47,000
Isoelectric point, pH	5.6	8.6	7.8
Sedimentation coefficient, S	5.8	4.7	3.5

DNA polymerase α and the C1 and C2 proteins were resolved from DNA polymerase α_2 by successive steps of hexylagarose and phosphocellulose chromatography by the procedure described in the text. Molecular weights under nondenaturing conditions were estimated from the R_M values relative to molecular weight marker proteins after electrophoresis on 5%, 7%, and 8% crosslinked polyacrylamide gels. Subunit structure was determined by $NaDodSO_4$/polyacrylamide gel electrophoresis on 5%, 7%, and 10% crosslinked gels according to the procedure of Laemmli (28). Sedimentation coefficients were determined by centrifugation in 10–30% glycerol gradients according to published procedures (13, 21). Isoelectric points were determined by isoelectric focusing according to published procedures (30).

Table 2. Template utilization by DNA polymerase α_2 and α

Template-primer	dNMP incorporated by DNA polymerases, pmol: α_2	α
Activated DNA	618	380
Denatured DNA	661	15
Native DNA	12	6
Hydroxylapatite isolated:		
Single-strand DNA	<2	<2
Loop-back DNA	509	11
Exonuclease III-treated DNA	471	<2
Poly(dA)·$(dT)_{12-18}$		
20:1	231	215
40:1	430	87
80:1	791	91

DNA polymerase α is the fraction of DNA polymerase α_2 eluted from hexylagarose by 0.5% Triton X-100. DNA polymerase α_2 was the fraction from DEAE-Bio-Gel chromatography eluted by 0.1 M KCl. DNA polymerase assays with DNA templates were performed with 5 μg of the respective templates and 2.2 units of polymerase α or 1.3 units of polymerase α_2 per assay. The template-to-primer ratio for poly(dA)·$(dT)_{12-18}$ was 20:1, 40:1, or 80:1 (in mononucleotide concentration).

The DNA polymerase α activity derived from successive steps of chromatography of DNA polymerase α_2 on hexylagarose and phosphocellulose does not effectively utilize DNA templates that contain extensive single-strand regions (Table 2). This is apparently due to the separation of the C1 and C2 factors from the DNA polymerase α during the hydrophobic affinity and phosphocellulose chromatographic steps. Recombination of the separated C1 and C2 factors with polymerase α reconstituted about 75% of its activity with single-strand DNA templates (Table 3). Both the C1 and C2 factors are required in addition to DNA polymerase α for reconstitution of this catalytic activity of DNA polymerase α_2.

C1 and C2 Factors Are Not DNases. The purified C1 and C2 factors do not have detectable exodeoxyribonuclease activity when assayed with ^{3}H-labeled native or denatured *E. coli* DNA or [^{3}H]poly(dT). They also show no detectable endonucleolytic activity when assayed with SV40 form I [^{3}H]DNA or bacteriophage fd [^{3}H]DNA. In addition, there were no measurable DNA polymerase α, β, or γ, RNA polymerase (31), or DNA-

Table 3. Requirement of C1 and C2 factors for synthesis by DNA polymerase α with single-strand DNA template-primer

Enzyme and factors	dNMP incorporated, pmol: Activated DNA	Loop-back DNA
DNA polymerase α_2	503	553
DNA polymerase α	460	6
DNA polymerase α plus:		
C1 factor	469	31
C2 factor	476	13
C1 plus C2 factors	492	427

DNA polymerase α_2 was the fraction eluted from DEAE-Bio-Gel with 0.1 M KCl that was rechromatographed on denatured DNA-cellulose and DEAE-Bio-Gel. DNA polymerase α was the fraction from hexylagarose chromatography of DNA polymerase α_2 that was eluted by 0.5% Triton X-100 and further purified by phosphocellulose chromatography. The C1 and C2 factors were the 0.5 M potassium phosphate-eluted fractions from phosphocellulose chromatography of the 1% and 0.5% Triton X-100 eluates from hexylagarose chromatography of DNA polymerase α_2. DNA polymerase was assayed with 1.2 units of DNA polymerase α or α_2 and 1 μg each of C1 and C2 factor where designated.

dependent or independent ATPase (32) activities with the purified C1 and C2 factors.

The C1 and C2 factors are thought to be proteins on the basis of their sensitivity to protease, insensitivity to DNase and RNase, and their molecular size (unpublished data).

DISCUSSION

In an earlier report we described a protein factor (C1) from HeLa cells that specifically stimulated HeLa cell DNA polymerase α activity 20-fold with DNA templates that contain extensive single-strand regions (13). In this report we show a cofractionation of the C1 factor and an additional protein factor (C2) with one of three chromatographic forms of DNA polymerase α from synchronized HeLa cells. The factors cofractionate with the DNA polymerase during chromatography and rechromatography on native and denatured DNA-cellulose, DEAE-Bio-Gel, gel filtration on Bio-Gel A-1.5m in the presence of 0.5 M KCl, and nondenaturing polyacrylamide gel electrophoresis. However, the C1 and C2 factors are resolved from the DNA polymerase by the combined steps of hydrophobic affinity chromatography on hexylagarose and phosphocellulose chromatography.

Both the C1 and C2 factor are required in order for the DNA polymerase α to function with single-strand templates. The polymerase α separated from the C1 and C2 factors has negligible activity with DNA templates that contain extensive single-strand regions. The requirement by DNA polymerase α for C1 and C2 factors appears to be limited to its activity with single-strand DNA templates.

The elution profile of HeLa cell DNA polymerase α on DEAE-Bio-Gel and the recovery of its activity as DNA polymerases α_1, α_2, and α_3 in this study is in agreement with observations of Johnston and coworkers (2) on the resolution of DNA polymerase α from calf thymus as the A_1, A_2, and C forms. DNA polymerases α_1, α_2, and α_3 from HeLa cells have sedimentation coefficients of 8.9S, 8.7S, and 7S, respectively in 10–30% glycerol gradients in the presence of 0.5 M KCl. The molecular weights of DNA polymerases α_2 and α_3 as determined by nondenaturing polyacrylamide gel electrophoresis are 640,000 and 220,000, respectively. $NaDodSO_4$/polyacrylamide gel electrophoresis of DNA polymerase α_2 previously purified by nondenaturing polyacrylamide gel electrophoresis yielded a protein band of 140,000 molecular weight and four bands of lower molecular weight materials of 69,000, 55,000, 47,000, and 24,500. $NaDodSO_4$/polyacrylamide gel electrophoresis of DNA polymerase α_3 further purified by nondenaturing gel electrophoresis yielded two protein bands of 140,000 daltons and 65,000 daltons. $NaDodSO_4$/polyacrylamide gel electrophoresis of the DNA polymerase derived from DNA polymerase α_2 by chromatography on hexylagarose and phosphocellulose yielded a single protein band of 140,000 daltons. Thus, the 140,000-dalton protein appears to be the basic catalytically active subunit.

The molecular weight of the catalytic subunit of the α polymerase from HeLa cells is similar to that which has been reported, in certain cases, for the catalytic subunit of the α polymerase from calf thymus (2, 11, 12), rat liver (8), and *Drosophila melanogaster* embryos (7, 9). It is of interest that in these cases also the activity of the catalytic subunit is dependent upon lower molecular weight proteins that are separated during the resolution of the α polymerase.

Several different protein factors have now been reported that stimulate DNA polymerase α activity *in vitro* with primed single-strand DNA templates (13, 33, 34). Also, it was recently reported that DNA polymerase β in addition to polymerase α is required for *in vitro* synthesis of full-length linear DNA strands of bacteriophage ϕX174 DNA templates containing an RNA primer (35). It seems probable that a number of proteins in addition to polymerase α are required for the elongation step of DNA replication in eukaryotes as has already been clearly demonstrated in prokaryotes (36).

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1. Momparler, R. L., Rossi, M. & Labitan, A. (1973) *J. Biol. Chem.* **248,** 285–293.
2. Holmes, A. M., Hesselwood, I. P. & Johnston, I. R. (1974) *Eur. J. Biochem.* **43,** 487–499.
3. Craig, R. K. & Keir, H. (1975) *Biochem. J.* **145,** 225–232.
4. Matsukage, A., Bohn, E. W. & Wilson, S. H. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 578–582.
5. Hachmann, J. J. & Lezius, A. G. (1975) *Eur. J. Biochem.* **50,** 357–366.
6. Fisher, P. & Korn, D. (1977) *J. Biol. Chem.* **252,** 6528–6535.
7. Banks, G. R., Boezi, J. A. & Lehman, I. R. (1979) *J. Biol. Chem.* **254,** 9886–9892.
8. Mechali, M., Abadiedebat, J. & De Recondo, A.-M. (1980) *J. Biol. Chem.* **255,** 2114–2122.
9. Villani, G., Saver, B. & Lehman, I. R. (1980) *J. Biol. Chem.* **255,** 9479–9483.
10. Chen, Y.-C., Bohn, E. W., Planck, S. R. & Wilson, S. H. (1979) *J. Biol. Chem.* **254,** 11678–11687.
11. Holmes, A. M., Hesselwood, I. P. & Johnston, I. R. (1976) *Eur. J. Biochem.* **62,** 229–235.
12. McKune, K. & Holmes, A. M. (1979) *Nucleic Acids Res.* **6,** 3341–3362.
13. Novak, B. & Baril, E. F. (1978) *Nucleic Acids Res.* **5,** 221–239.
14. Marmur, J. (1961) *J. Mol. Biol.* **3,** 208–218.
15. Baril, E., Mitchener, J., Lee, L. & Baril, B. (1977) *Nucleic Acids Res.* **4,** 2641–2653.
16. Wilson, D. A. & Thomas, C. A. (1973) *Biochim. Biophys. Acta* **331,** 333–340.
17. Kelly, R. & Sinsheimer, R. (1967) *J. Mol. Biol.* **29,** 229–242.
18. Kornberg, T. & Gefter, M. L. (1972) *J. Biol. Chem.* **247,** 5369–5375.
19. Sadowski, P. D. & Hurwitz, J. (1969) *J. Biol. Chem.* **244,** 6192–6198.
20. Alberts, B. & Herrick, G. (1971) *Methods Enzymol.* **21,** 198–217.
21. Chiu, R. C. & Baril, E. F. (1975) *J. Biol. Chem.* **250,** 7951–7957.
22. Baril, E. F., Jenkins, M. D., Brown, O. E., Laszlo, J. & Morris, H. P. (1973) *Cancer Res.* **33,** 1187–1193.
23. Knopf, K. W., Yamada, M. & Weissbach, A. (1976) *Biochemistry* **15,** 4540–4548.
24. Ohashi, M., Taguchi, T. & Ikegami, S. (1978) *Biochem. Biophys. Res. Commun.* **82,** 1084–1090.
25. Wright, G. E., Baril, E. F. & Brown, N. C. (1980) *Nucleic Acids Res.* **8,** 99–105.
26. Edenberg, H. J., Anderson, S. & DePamphilis, M. L. (1978) *J. Biol. Chem.* **253,** 3278–3280.
27. Waqar, M. A., Evans, M. J. & Huberman, J. A. (1978) *Nucleic Acids Res.* **5,** 1933–1946.
28. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
29. Schaffner, W. & Weissman, C. (1973) *Anal. Biochem.* **56,** 502–514.
30. Vesterberg, O., Hansen, L. & Sjösten, A. (1977) *Biochim. Biophys. Acta* **49,** 160–166.
31. Roeder, R. G. & Rutter, W. J. (1970) *Biochemistry* **9,** 2543–2553.
32. Assairi, L. & Johnston, I. (1979) *Eur. J. Biochem.* **99,** 71–79.
33. Otto, B., Baynes, M. & Knippers, R. (1977) *Eur. J. Biochem.* **73,** 14–17.
34. Burke, J., Plummer, J., Huberman, J. & Evans, M. J. (1980) *Biochim. Biophys. Acta* **609,** 205–223.
35. Ikeda, J.-E., Longiaru, M., Horwitz, M. S. & Hurwitz, J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5827–5831.
36. Kornberg, A. (1980) *DNA Synthesis* (Freeman, San Francisco).