and double-strand cuts in native DNA is interesting in this respect and also may indicate involvement in other processes. It has been suggested that the separation of long linear eukaryotic chromosomes, which may be topologically interlocked, might require the mediation of enzymes able to cleave and rejoin duplex DNA (Hsieh & Brutlag, 1980). The nuclease described in this work could represent the nucleolytic subunit or part of a multienzyme complex able to perform such a function. Further investigation into the conditions and mode of action of this endonuclease will clarify its role within the cell nucleus.

Acknowledgments

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Purification of a 9S DNA Polymerase α Species from Calf Thymus†

Frank Grosse and Gerhard Krauss*

ABSTRACT: A DNA polymerase α species from calf thymus has been purified 12 000-fold to near homogeneity. The enzyme sediments under high salt conditions in the preparative ultracentrifuge as a homogeneous band at 9 S. The specific activity is 50 000–70 000 units/mg of protein. Polypeptides of 148 000, 59 000, 55 000, and 48 000 daltons are detectable. The molecular weight as estimated from gradient gel electrophoresis is about 500 000. The 9S DNA polymerase is free from terminal nucleotidyl transferase activity and does not exhibit endonuclease or exonuclease activity. It is inhibited by low concentrations of salt, aphidicolin, and *N*-ethylmaleimide.

The purification and characterization of DNA polymerases have proven to be a difficult task. The subunit structure of DNA polymerase III holoenzyme—the main replicative enzyme in *Escherichia coli*—has been established only in the last few years [for a review, see Kornberg (1980)]. Whereas the polymerizing activity of the core subunit of this enzyme has been discovered rather early (Otto et al. 1973), little is known up to now about the function of the other subunits.

In mammals, DNA polymerase α is responsible for the

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replication of the cellular DNA. Although DNA polymerase α has been the subject of numerous investigations, its subunit structure has remained controversial. In some organisms and tissues, a 150 000-dalton catalytic core enzyme that is associated with subunits in the range of 50 000–70 000 daltons could be isolated (Holmes et al., 1976; Méchali et al., 1980; Banks et al., 1979). On the contrary, DNA polymerase α has also been reported to consist only of 50 000–70 000-dalton subunits (Fisher & Korn, 1977; Grummt et al., 1979; Chen et al., 1979).

Recently we have described the purification of a 5.7S DNA polymerase α from calf thymus (Grosse & Krauss, 1980). In the present paper, we report the isolation and partial characterization of a DNA polymerase α that sediments at 9 S. The 9S polymerase contains subunits of 148 000, 59 000, 55 000, and 48 000 daltons. We have evidence that the 148-kilodalton subunit carries enzymatic activity and that partial degradation of this polypeptide can lead to the appearance of smaller polymerase α species.

Materials and Methods

Materials

Unlabeled deoxyribonucleotides were purchased from Boehringer-Mannheim. Poly- and oligonucleotides were from Collaborative Research. [^{3}H]dTTP was from Schwarz Mann, and [^{3}H]poly(dA) was from Miles. DEAE-cellulose DE 23 and phosphocellulose P 11 were purchased from Whatman. Calf thymus DNA (grade I) was from Sigma. Plasmid pBR 322 was a kind gift from J. Langowski of our laboratory. Sepharose 4B CL and BrCN-activated Sepharose were from Pharmacia. Bio-Rex 70 was from Bio-Rad. Coupling of heparin and DL-valine to BrCN-activated Sepharose 4B CL was performed according to the instructions of Pharmacia. Hydroxylapatite was prepared according to Bernardi (1969). Bovine pancreatic DNase, bovine liver catalase, rabbit muscle L-lactate dehydrogenase, and bovine serum albumin were from Boehringer-Mannheim. *E. coli* RNA polymerase was a gift from Dr. J. Hogget, and *E. coli* elongation factor TU was a gift from Dr. A. Pingoud of our laboratory. Trasylol was from Bayer (Germany). Heparin and DL-valine were from Serva. Phenylmethanesulfonyl fluoride (PMSF)[1] was purchased from Merck and was stored as a stock solution (10^{-1} M) in 2-propanol. Aphidicolin was a gift from ICI. Ampholine (pH 3.5–10) was from LKB. All other chemicals were of the highest purity available from commercial sources. Activated calf thymus DNA (20% acid soluble) was prepared as prescribed by Baril et al. (1977).

Methods

DNA Polymerase Assays. The assay (50 μL) contained 60 mM Tris-HCl, pH 7.8, 5 mM $MgCl_2$, 1 mM dithioerythritol, 0.3 mg/mL bovine serum albumin, 0.5 mg/mL activated DNA, 0.8% Ampholine, 100 μM each of dATP, dGTP, dCTP, and [^{3}H]dTTP (5 cpm/pmol), and appropriately diluted enzyme (5 μL). Following incubation for 5 min at 37 °C, an aliquot was pipetted on a Whatman GFC filter disk. The incorporation of labeled nucleotides into acid-insoluble product was measured as outlined by Fasiolo et al. (1970). One unit of polymerase α activity was defined as the amount of enzyme required to convert 1 nmol of dTTP into acid-precipitable product in 1 h at 37 °C. Assays using poly(dA)·$(dT)_{10}$ and poly(rA)·$(dT)_{10}$ as template-primers were carried out as described by Hesslewood et al. (1978) and Masaki & Yoshida (1978).

Other Enzyme Assays. RNA polymerase was assayed according to Burgess (1969). Terminal transferase was assayed according to Bollum et al. (1974) using $d(pT)_{40-60}$ as substrate.

Endonuclease activity was assayed by following the conversion of double-stranded superhelical plasmid pBR 322 to nicked or linear forms in the presence of DNA polymerase α. Analysis was performed in agarose gels with ethidium bromide staining. Nuclease activity was also measured by following the loss of acid-precipitable radioactivity from [^{3}H]poly(dA) in the presence of DNA polymerase α. The nuclease assays were performed at 37 °C in 10 mM Tris-HCl, pH 7.8, 50 mM KCl, 5 mM $MgCl_2$, and 1 mM 2-mercaptoethanol.

Electrophoresis. $NaDodSO_4$ electrophoresis was performed in 7.5% acrylamide slab gels by using the system of Laemmli (1970). Prior to electrophoresis, samples were precipitated with 10% Cl_3CCOOH and heated to 100 °C in Laemmli loading buffer. Nondenaturing gradient electrophoresis was run in 8 × 8 × 0.3 cm slab gels. A linear gradient of 3–10% acrylamide was applied from the bottom of the gel cassette. For control of the quality of the gradient, 0.3% bromophenol blue was included in the 3% acrylamide solution. Composition of the gel and prerunning and running buffers were the same as those given by Fisher & Korn (1977), except that the 3% stock solution contained twice the concentration of ammonium persulfate and *N,N,N′,N′*-tetramethylenediamine. Polymerization time was about 3 h. Prerunning, loading, and running of the gels were performed exactly as outlined by Fisher & Korn (1977).

Determination of Stoichiometries. Stoichiometries of subunits were determined from $NaDodSO_4$ gels after staining with Coomassie blue. Gels were photographed, and the negatives obtained were scanned in a Zeiss PMQ2 spectrophotometer. The scanning signal was stored in a digital recorder. Areas of the peaks were evaluated by a computer program (J. Langowski, unpublished experiments). The validity of the whole procedure was checked by evaluating the subunit composition of RNA polymerase (*E. coli*) that had been subjected to the same staining and photographing conditions as those of the DNA polymerase sample.

Ultracentrifugation. Preparative ultracentrifugation runs were carried out in the SB 283 rotor of an IEC B60 ultracentrifuge. Isokinetic gradients (12 mL per tube) were prepared with a constant-volume mixer (Noll, 1967). Sucrose concentration at the meniscus was 0.18 M. The mixing volume of the constant-volume mixer was 9.5 mL. The sucrose concentration in the reservoir was 0.92 M. The centrifuge was run at 40 000 rpm for 40 h. Tubes were pierced from the bottom, and fractions of 350 μL were collected. Sedimentation equilibrium runs were carried out in a Spinco Model E ultracentrifuge. Preparative and analytical ultracentrifugation runs were performed in 30 mM potassium phosphate, pH 7.8, 0.4 M KCl, 0.5% Ampholine, 1 mM disulfite, 1 mM EDTA, and 7 mM 2-mercaptoethanol at 4 °C.

Protein concentration was determined according to Lowry et al. (1951). For the pure 9S enzyme, the protein concentration was determined by assuming $A_{280nm}^{1mg/mL}$ to be equal to 1.

Results and Discussion

Preparation of the 9S Polymerase α. All operations are performed at 4 °C. A purification scheme is outlined in Table I. All buffers contain 10 mM disulfite, 14 mM 2-

[1] Abbreviations used: EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; NEM, *N*-ethylmaleimide; PMSF, phenylmethanesulfonyl fluoride; $NaDodSO_4$, sodium dodecyl sulfate; Cl_3CCOOH, trichloroacetic acid.

Table I: Purification of DNA Polymerase α from Calf Thymus[a]

fraction		total activity units ($\times 10^{-3}$)	sp act. (units/mg)	yield (%)
I	crude extract	500	5	100
II	DEAE batch	300	50	60
III	phosphocellulose batch	220	250	44
IV	heparin-Sepharose	110	500	22
V	valyl-Sepharose	80	3000	16
VI	hydroxylapatite	60	9000	12
VII	Bio-Rex 70	50	14000	10
VIII	ultracentrifugation	30	60000	6

[a] One unit corresponds to the incorporation of 1 nmol of dTTP in 1 h at 37 °C with activated DNA as substrate. The purification scheme refers to 1 kg of calf thymus.

mercaptoethanol, and 1 mM EDTA.

Preparation of the Crude Extract. Thymus glands are collected from freshly slaughtered calves and stored at −80 °C. Glands (1000 g) are allowed to thaw overnight and are homogenized in 8 L of 10 mM potassium phosphate, pH 8.2, in a Waring blendor (2 min at low speed). The homogenate is centrifuged for 45 min at 9000 rpm in the Sorvall GS3 rotor to remove debris. The supernatant fluid is poured through eight layers of cheesecloth (fraction I).

DEAE-cellulose and Phosphocellulose Batch Elution. The crude extract is stirred for 30 min with 3 L of DEAE-cellulose DE 23 (equilibrated in 10 mM potassium phosphate, pH 8.2). The DE 23 is filtered through a large funnel with suction and washed in the funnel with 15 L of 10 mM potassium phosphate, pH 8.2. More than 90% of the DNA polymerase activity binds to the DE 23 in this step. Elution is carried out with 5 L of 0.2 M potassium phosphate, pH 8.0. Fractions of 400 mL are collected, and those containing activity (1800 mL, fraction II) are combined. After dilution with 1 volume of distilled water, the pH is adjusted to 7.2, and 500 mL of phosphocellulose P 11 (equilibrated in 80 mM potassium phosphate, pH 7.2) is added. The suspension is stirred for 30 min and filtered through a funnel. The phosphocellulose is washed in the funnel with 3 L of 80 mM potassium phosphate, pH 7.2, and DNA polymerase activity is eluted with 2 L of 0.25 M potassium phosphate, pH 7.2. Active fractions (600 mL) are diluted with 3 volumes of distilled water to yield fraction III.

Heparin–Sepharose Chromatography. Fraction III is passed through a column (5 × 15 cm) of heparin–Sepharose at a flow rate of 300 mL/H. The column is washed with 1 volume of 80 mM potassium phosphate, pH 7.8. A gradient of 0.08–0.3 M potassium phosphate (1500 mL of each) is applied. DNA polymerase α elutes at about 0.12–0.14 M potassium phosphate, together with terminal transferase activity (fraction IV).

Valyl-Sepharose Chromatography. A saturated ammonium sulfate solution (adjusted to pH 7.2 with Tris base) is pumped into fraction IV at a rate of 30 mL/h to yield 33% saturation. The precipitate is removed by centrifugation and the supernatant applied to a valyl-Sepharose column (1.5 × 10 cm) equilibrated in 50 mM potassium phosphate, pH 7.8, and 33% ammonium sulfate. The column is developed with a gradient of descending ammonium sulfate concentration (33–10% saturation, 250 mL each). DNA polymerase α elutes at about 25% ammonium sulfate saturation. Fractions containing DNA polymerase were combined to yield fraction V.

Hydroxylapatite Chromatography. Saturated ammonium sulfate solution is added to fraction V to yield 60% saturation. The precipitate is collected by centrifugation and is dissolved in 3 mL of 10 mM potassium phosphate and 0.5 M KCl. The solution is dialyzed for 4 h against 500 mL of 10 mM potassium phosphate, pH 7.8, and 0.5 M KCl and is passed through a hydroxylapatite column (0.9 × 8 cm). After being washed with 10 mL of the same buffer, a linear gradient of 10 mM potassium phosphate and 0.5 M KCl to 200 mM potassium phosphate, pH 7.8, and 0.5 M KCl (30 mL of each) is applied. DNA polymerase α elutes at about 100 mM potassium phosphate (fraction VI). This step removes residual terminal transferase activity that elutes before the DNA polymerase at about 0.07 M potassium phosphate.

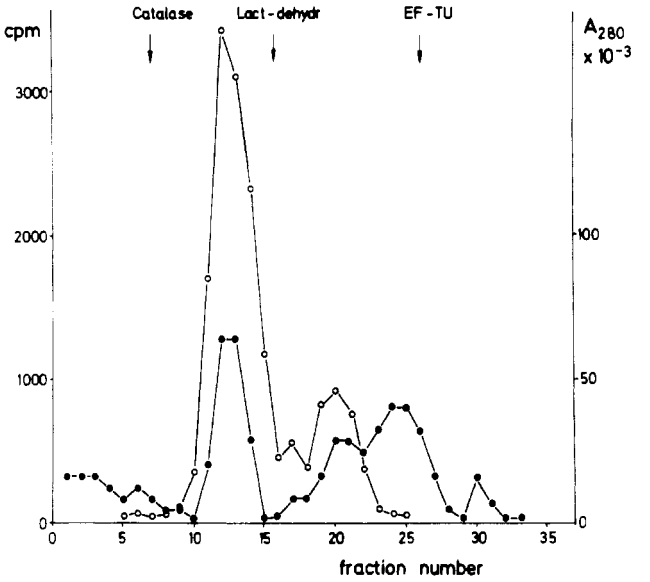

FIGURE 1: Preparative ultracentrifugation of the 9S enzyme. Fraction VII (see Table I) was run on an isokinetic sucrose gradient. For details, see Methods. (○) DNA polymerase α activity; (●) absorbance at 280 nm. Arrows indicate the positions of the reference proteins: catalase, 11.3 S; lactate dehydrogenase, 7.3 S; elongation factor TU (*E. coli*), 3.2 S.

Bio-Rex 70 Chromatography. Fraction VI is diluted with 6 volumes of distilled water and is passed through a Bio-Rex 70 column [0.9 × 8 cm, equilibrated in 70 mM potassium phosphate and 20% (v/v) ethylene glycol]. A gradient of 70–250 mM potassium phosphate elutes DNA polymerase α at about 150 mM potassium phosphate (fraction VII).

Preparative Ultracentrifugation. Fraction VII is precipitated by the addition of 3 volumes of saturated ammonium sulfate solution. After being stirred for 15 min, the solution is centrifuged at 10000*g* for 15 min. The precipitate is dissolved in 500 μL of 30 mM potassium phosphate, pH 7.8. The clear solution is dialyzed for 3 h against 50 mL of 30 mM potassium phosphate, pH 7.8, 0.4 M KCl, and 0.5% Ampholine. The dialysate is applied on top of the gradient in 150-μL portions. Figure 1 shows the results of an ultracentrifugation run. The main portion of polymerase α sediments at 9 S as a distinct optical density peak. Two smaller polymerase species are also detectable that sediment at 7.0–7.5 S and 5.7–6.0 S. It should be pointed out that it is also possible to obtain preparations where only 9S enzyme is present (see below).

The fractions of the 9S activity peak are diluted 3-fold with distilled water and are applied on a 0.5-mL Bio-Rex 70 column. The enzyme is eluted with 0.3 M potassium phosphate, pH 7.8, 20% (v/v) ethylene glycol, and 30% (w/v) sucrose and is stored in the same buffer at −20 °C.

Comments on the Purification Scheme. We have reproduced the purification scheme described above 8 times. We always end up predominantly with the 9S species. The amount

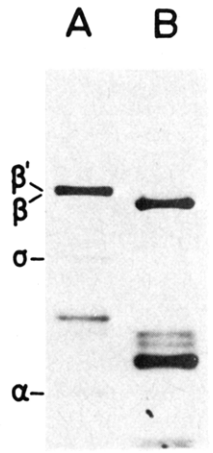

FIGURE 2: $NaDodSO_4$–polyacrylamide gel electrophoresis of the DNA polymerase activity peak sedimenting at 9 S. Fraction 13 (30 μg of protein) of the ultracentrifuge experiment shown in Figure 1 was subjected to electrophoresis in 7.5% Laemmli gels as outlined under Methods (B). The subunits of RNA polymerase (*E. coli*) were used as molecular weight markers (A): β' = 165 kilodaltons; β = 155 kilodaltons; σ = 95 kilodaltons; α = 39 kilodaltons.

of the smaller species depends on how fast the first steps of the purification scheme are carried out. None of the smaller species may be detectable if one arrives at fraction IV within 24 h and at fraction VII within 4 days after homogenization of the glands. The amount of 9S species finally obtained does not depend on whether the glands are homogenized immediately after slaughtering or whether they are kept frozen from homogenization. The inclusion of protease inhibitors like PMSF or aprotinin in the buffer did not noticeably change the yield of 9S enzyme; the addition of disulfite in all buffers, however, increases the fraction of 9S enzyme. A similar result has been reported by Banks et al. (1979). It is not always necessary to include the Bio-Rex 70 chromatography. If the specific activity of fraction VI is better than 12000 units/mg, the Bio-Rex step may be omitted. We have not been able to achieve a purification of the enzyme without preparative ultracentrifugation as the final purification step. It is mandatory to include Ampholine in the buffer of the ultracentrifugation run; otherwise, inactivation of the 9S enzyme will occur.

Structure of the 9S Enzyme. The 9S DNA polymerase α migrates as a homogeneous, distinct band in the preparative ultracentrifuge runs (Figure 1). The specific activity of 50000–70000 units/mg is constant over the optical density peak. $NaDodSO_4$ electrophoresis reveals subunits of 148, 59, 55, and 48 kilodaltons (Figure 2). This pattern is the same for all fractions of the activity peak. The stoichiometric ratio of the subunits as obtained from a scan of a photograph of the $NaDodSO_4$ gel is about 1:1:1:5 (see Figure 3). Thus, the 48-kilodalton subunit is present in excess over the other subunits. Sometimes we observed slight variations in this subunit structure. In two preparations of the 9S enzyme, we did not observe the 55-kilodalton subunit. Instead, a 74-kilodalton polypeptide showed up. At present, we do not know the reason for this change in the pattern of the small subunits. We suspect as yet uncontrolled proteolytic events to be responsible for the varying subunit pattern. Holmes et al. (1976) also have reported the presence of a 70-kilodalton polypeptide in highly purified polymerase α preparations from calf thymus.

The structure of the 9S enzyme from calf thymus is very similar to that of the DNA polymerase α from *Drosophila* (Banks et al., 1979). This enzyme has been found to consist of subunits of 148, 58, 46, and 43 kilodaltons. Mêchali et al. (1980) have described a polymerase α from rat liver, the

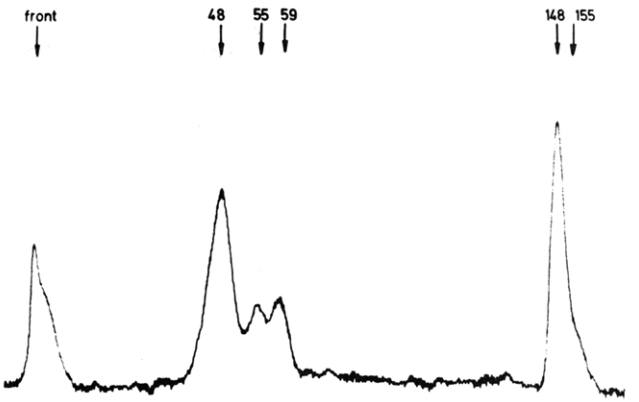

FIGURE 3: Scan of the $NaDodSO_4$ gels of the 9S activity peak. $NaDodSO_4$ gels were photographed and scanned. Stoichiometries of the polypeptides were determined by relating the area under the peak to the molecular weights indicated above the scan (see Methods).

subunit composition of which also bears similarities to that of the 9S enzyme from calf thymus. However, for both the *Drosophila* and the rat liver enzyme, the stoichiometry of the subunits is still unclear. In both systems, at least one of the smaller subunits is present in excess over the other ones.

In all preparations of the 9S enzyme, we observed a polypeptide of 158 kilodaltons, representing about 5–10% of the staining intensity of the 148-kilodalton subunit. It is interesting to note that a polypeptide of about 158 kilodaltons is visible in the gels of the *Drosophila* enzyme too (Banks et al., 1979) and that the large subunit of the rat liver enzyme has a molecular weight of 159 kilodaltons. The relation of the 158-kilodalton polypeptide to the 148-kilodalton polypeptide in the calf thymus system is not yet clear. We do not know whether the 158-kilodalton polypeptide bears catalytic activity or whether it is just a contamination.

Native Molecular Weight of the 9S Enzyme. Information about the native molecular weight of the 9S enzyme has been obtained up to now only from native gradient electrophoresis. From 3 to 10% gradient gels, a native molecular weight of about 500 kilodaltons is determined. However, this number gives only a rough idea about the true native molecular weight, since there is a strong influence of the shape of the molecule on the mobility in the gels. Attempts to determine the native molecular weight from sedimentation equilibrium runs in the analytical ultracentrifuge have failed up to now. Molecular weights evaluated from runs at 8000 rpm were in the range of 70–150 kilodaltons, which is far too low for the sedimentation coefficient of 9 S. $NaDodSO_4$ gel analysis at the end of the ultracentrifuge run showed that the subunits had not been degraded by proteolysis. However, only about 5% of the initial activity was recovered. Possibly denaturation is responsible for the dissociation of the subunits and the loss of activity.

Relation of the 9S Enzyme to the Other Polymerase α Species in Calf Thymus. We have recently reported the purification of a 5.7S DNA polymerase α species from calf thymus. This enzyme is obtained as an equimolar mixture of polypeptides of 134 and 123 kilodaltons. A polymerase α species sedimenting at about 5.7–6.0 S is also detectable in the final ultracentrifugation step of the purification of the 9S enzyme. As already pointed out, the amount of 5.7S species depends on how fast the first steps of the purification are carried out. However, the 5.7S enzyme obtained this way is not pure. Besides of 134- and 123-kilodalton polypeptide, other polypeptides of higher and lower molecular weights are present in the 5.7S activity peak. These are not identical with the subunits of the 9S enzyme and are considered as contaminants.

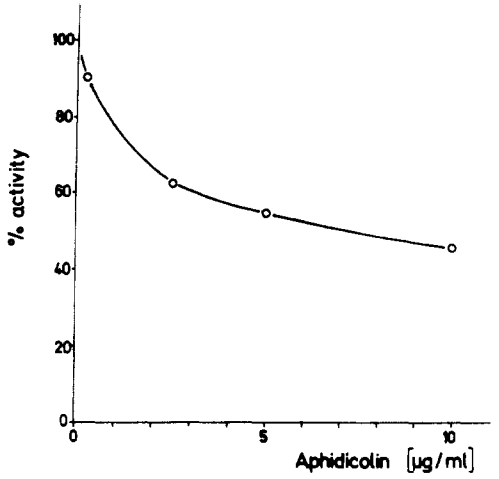

FIGURE 4: Inhibition of the 9S DNA polymerase by aphidicolin. The enzyme (0.1 μg) was assayed in the standard mixture with the inhibitor concentrations given. The template was activated DNA.

We have not been able to purify to homogeneity the polymerase sedimenting at 7.0–7.5 S. Besides other polypeptides, this activity peak contains the 148-kilodalton polypeptide and also polypeptides of the same size as that of the smaller subunits of the 9S enzyme. We consider the 5.7S enzyme to be a degradation product of the 9S enzyme. This conclusion is derived from two observations: (1) The amount of 9S enzyme obtained in the preparative ultracentrifugation step is inversely related to the amount of 5.7S enzyme. (2) In one sample of the 9S enzyme, we observed (after 48 h after 4 °C) a degradation of the 148-kilodalton subunit to a series of smaller polypeptides. Among these were 140-, 134-, 123-, 110-, and 90-kilodalton species (gels not shown).

Presumably, the 134- and 123-kilodalton polypeptides are identical with those of the 5.7S enzyme. Evidently, this preparation still contained some protease activity that led to the degradation of the 148-kilodalton subunit. These results and the detection of 134- and 123-kilodalton polypeptides in a pure 5.7S polymerase (Grosse & Krauss, 1980) strongly suggest that the 148-kilodalton subunit of the 9S enzyme carries the enzymatic activity. Evidently, the 148-kilodalton subunit can be partially degraded to polypeptides that do not interact with the smaller subunits but are still active in in vitro DNA synthesis.

It has been reported that DNA polymerase α consists of subunits of 60–40 kilodaltons only (Grummt et al., 1979). Our results clearly are not in agreement with these findings. Possibly, proteolysis interfered with the polymerase preparation of Grummt et al.

Enzymatic Properties of the 9S Enzyme. The 9S polymerase exhibits most of the properties that have been found to be typical for DNA polymerase α. The 9S enzyme is strongly inhibited by aphidicolin (Figure 4). The inhibition is slightly less than that observed by Banks et al. (1979) for the enzyme from *Drosophila*. Aphidicolin has been demonstrated to be a specific inhibitor of mammalian replicative polymerases (Ikegami et al., 1978). *N*-Ethylmaleimide (NEM) also inhibits the enzyme strongly. Incubation of the 9S polymerase with 0.01 mM NEM for 10 min at 37 °C destroys half of the activity. The NEM sensitivity of the 9S enzyme is considerably greater than that of the 8S enzyme reported by Wickremasinghe et al. (1977); it is comparable, however, to that of the *Drosophila* enzyme (Banks et al., 1979).

The 9S polymerase is remarkably stable at elevated temperatures. Incubation of the enzyme for 7 h at 37 °C in the assay buffer leads to a loss of activity of only 6%. At 50 °C, half of the activity is lost within 5 min (Figure 5). The heat denaturation profile is different from that reported for the rat liver enzyme (Méchali & Recondo, 1980), where a biphasic behavior was observed.

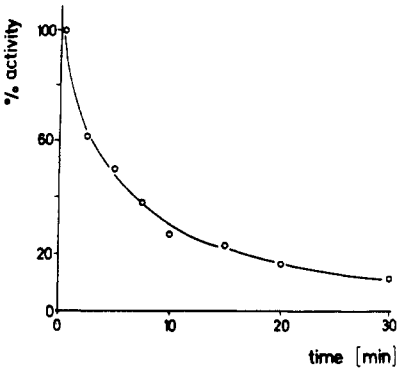

FIGURE 5: Heat inactivation. Fraction VIII (1 μg) was incubated at 50 °C in 20 mM KP_i pH 7.8, 5% ethylene glycol, 5% sucrose, 0.2 mM EDTA, and 1 mM 2-mercaptoethanol. Aliquots were withdrawn at the time given and were assayed in the standard mixture.

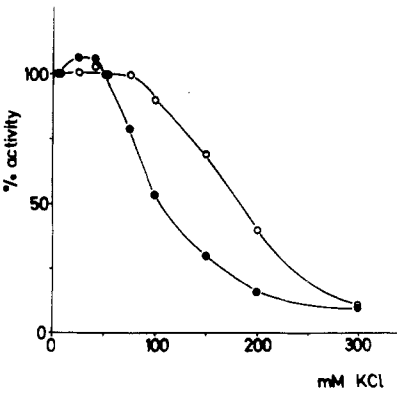

FIGURE 6: Salt concentration dependence of 9S and 5.7S DNA polymerase α: (○) 5.7S enzyme; (●) 9S enzyme. Enzyme (0.1 μg) were assayed in the standard mixture with the salt concentrations indicated and with 0.8% Ampholine present. The 5.7S enzyme was purified as outlined by Grosse & Krauss (1980).

Table II: Template-Primer Utilization of the 9S DNA Polymerase α[a]

template-primer	dTTP incorporated (pmol/30 min)
activated calf thymus DNA	520
denatured activated calf thymus DNA	370
poly(dA)·$(dT)_{10}$ (10:1)	75
poly(rA)·$(dT)_{10}$ (10:1)	1
pBR 322,[b] supercoiled	4

[a] 9S enzyme (1 unit) was assayed as described under Methods. The temperature was 37 °C for DNA as substrate and 30 °C for the homopolymers. [b] pBR 322 (10 μg) was assayed with an amount of enzyme that incorporates 500 pmol of dTTP/30 min when 10 μg of activated DNA is present in the standard assay.

The enzyme prefers activated DNA to denatured activated DNA or poly(dA)·oligo$(dT)_{10}$ (Table II). We also can exclude the presence of terminal nucleotidyl transferase and RNA polymerase activity. The exclusion limit for both enzymes is 1×10^{-5}. The salt concentration dependence of the 9S enzyme is shown in Figure 6 together with the data already reported for the pure 5.7S enzyme (Grosse & Krauss, 1980). The two enzyme species differ in salt concentration dependence, the 9S enzyme being more sensitive to salt concentration than the 5.7S enzyme. The salt concentration sensitivity of DNA polymerase α is due to the weak binding to DNA

(Momparler et al., 1973). Presumably, the presence of the small subunits in the 9S enzyme leads to a weaker binding to DNA as compared to that of the 5.7S enzyme. The higher salt concentration sensitivity of the 9S enzyme goes in parallel with a reduced affinity of the 9S enzyme to heparin–Sepharose. The 9S enzyme elutes at 0.12–0.14 M potassium phosphate; the 5.7S enzyme, however, elutes at about 0.16 M potassium phosphate (Grosse & Krauss, 1980).

Nuclease Activity. We were not able to detect exonuclease activity with an exonuclease/polymerase exclusion limit of 3×10^{-5}. Only traces of endonuclease activity are present in our 9S enzyme. It is estimated from the digestion experiments with pBR 322 as substrate that the ratio of endonucleolytic to polymerizing activity is less than 1 to 10^7. Thus, the 9S enzyme is clearly distinct from the δ polymerase from calf thymus that has been reported to contain nuclease activity (Tsang Lee et al., 1980).

Conclusions

A 9S DNA polymerase α from calf thymus has been purified to near homogeneity. The subunit composition and the enzymatic properties of the 9S enzyme are strikingly similar to those of the DNA polymerase α from *Drosophila* (Banks et al., 1979; Villani et al., 1980). The close similarity suggests that a common structural principle of polymerase α exists in eukaryotes. Furthermore, it is remarkable that the replicative enzyme in *E. coli* also consists of a 140-kilodalton catalytic core subunit which is associated with subunits of smaller size.

The heterogeneity of DNA polymerase α that is frequently observed could be due to two reasons: First, proteolysis of the large subunit can lead to smaller polypeptides that are still enzymatically active. Second, a partial loss of a smaller subunit can lead to a change in the chromatographic properties and the sedimentation behavior (Holmes et al., 1975). Polymerase α species of similar sedimentation behavior as that reported in the present paper have been observed in the *Drosophila* system too (Brakel & Blumenthal, 1977; Banks et al., 1979).

The exact stoichiometry and the function of the small subunits are not yet clear. The small variations in the subunit structure of the 9S enzyme could not yet be correlated with changes in the enzymatic activity. Since the activity of the 9S enzyme shows an increased salt concentration sensitivity as compared to that of the 5.7S enzyme, we suspect that the small subunits influence the enzyme–DNA interaction. Further work will have to clarify the function of the small subunits in the replication process.

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