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A Novel Primase-Free Form of Murine DNA Polymerase α Induced by Infection with Minute Virus of Mice†

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ABSTRACT: Two species of DNA polymerase α free of primase activity were identified in extracts of Ehrlich mouse cells that had been infected with minute virus of mice. Primase-free forms of DNA polymerase α eluted with 150 and 180 mM NaCl during ion-exchange chromatography on DEAE-cellulose columns, exhibited sedimentation coefficients of 11 S and 8.2 S, respectively, and were inhibited by aphidicolin, N^2-(*p*-*n*-butylphenyl)-9-(2-deoxy-β-D-ribofuranosyl)guanine 5′-triphosphate, and 2-(*p*-*n*-butylanilino)-9-(2-deoxy-β-D-ribofuranosyl)adenine 5′-triphosphate. The ratio of primase-free DNA polymerase α to the DNA polymerase α–primase complex increased from 1.5 to >100 during the course of infection, and free primase was produced during the MVM replicative cycle.

The eucaryotic replicative enzyme DNA polymerase α is characterized by its relatively high molecular weight, complex subunit composition, specific induction during the S-phase of the cell cycle, ability to use RNA primers, and sensitivity to aphidicolin, BuPdGTP,[1] and BuAdATP (Fry & Loeb, 1986). Highly purified preparations of DNA polymerase α from mammalian cells and insects as well as the analogous DNA polymerase I in yeast consist of a DNA polymerase catalytic subunit of M_r = 145 000–185 000 in tight association with two to three additional subunits of M_rs = 47 000–86 000 (Kaguni et al., 1983; Faust et al., 1984; Chang et al., 1984; Karawya et al., 1984; Wahl et al., 1984; Denhardt & Faust, 1985; Plevani et al., 1985; Vishwanatha et al., 1986; Holmes et al., 1986; Wong et al., 1986; Nasheuer & Grosse, 1987; Cotterill

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[1] Abbreviations: BuPdGTP, N^2-(*p*-*n*-butylphenyl)-9-(2-deoxy-β-D-ribofuranosyl)guanine 5′-triphosphate; BuAdATP, 2-(*p*-*n*-butylanilino)-9-(2-deoxy-β-D-ribofuranosyl)adenine 5′-triphosphate; PMSF, phenylmethanesulfonyl fluoride; BSA, bovine serum albumin; DTT, dithiothreitol.

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et al., 1987; Pausch et al., 1988). Usually DNA polymerase α preparations exhibit a tightly bound oligoribonucleotide polymerase called primase that is associated with the M_r = 47 000–70 000 polypeptides (Plevani et al., 1985; Holmes et al., 1986; Nasheuer & Grosse, 1987; Pausch et al., 1988). Primase synthesizes mixed oligoribo/deoxyribonucleotides of limited chain lengths (7–15 nucleotides) that are initiated exclusively with ATP or GTP and can be elongated in a coupled reaction catalyzed by the DNA polymerase α catalytic subunit (Conaway & Lehman, 1982; Yagura et al., 1983; Plevani et al., 1984; Chang et al., 1984; Gronostajski et al., 1984; Wang et al., 1984; Hu et al., 1984; Holmes et al., 1985; Grosse & Krauss, 1985; Faust et al., 1985; Yamaguchi et al., 1985; Singh et al., 1986). Genetic studies have revealed an essential role for primase and for the DNA polymerase α catalytic subunit in *Saccharomyces cerevisiae* (Johnson et al., 1985; Lucchini et al., 1987) and in mammalian cells (Murakami et al., 1985) where the human gene for the DNA polymerase α catalytic subunit has been mapped to the X chromosome (Wang et al., 1985). These physical, enzymological, and genetic properties, as well as a processivity of 100 nucleotides or less, are consistent with a role for the DNA polymerase α–primase complex in the discontinuous synthesis of RNA-primed DNA chains (Okazaki fragments) on the lagging strand of the DNA replication fork in eucaryotic cells (Prelich & Stillman, 1988; Stillman, 1988; Downey et al., 1988).

Minute virus of mice is an autonomous parvovirus belonging to the family Parvoviridae that has proven useful as a model system for probing cellular DNA replication mechanisms. Members of this virus family consist of icosahedral, nonenveloped particles that are 20 nm in diameter and contain single-stranded, linear DNA genomes (Tattersall & Cotmore, 1988). The MVM genome is 5149 nucleotides long (Sahli et al., 1985; Astell et al., 1986), with self-complementary sequences at the molecular termini that exist as stable hairpins (Astell et al., 1979, 1982, 1983). Viral DNA synthesis occurs in the nucleus (Singer & Rhode, 1978), is dependent on host cell factors expressed during the S-phase of the cell cycle (Tattersall, 1972; Wolter et al., 1980) and is catalyzed by host cell DNA polymerases. Three distinct phases of viral DNA replication are recognized including synthesis of the parental duplex DNA from the input viral single-stranded DNA, amplification of duplex RF DNA, and synthesis of progeny single-stranded DNA relatively late in the infectious cycle (Ward & Dadachanji, 1978; Faust & Gloor, 1984; Hauswirth, 1984). Initiation of viral DNA replication is self-primed at terminal palindromic sequences that fold into stable hairpin structures (Tattersall & Ward, 1976; Faust & Ward, 1979; Astell et al., 1982, 1985; Tattersall & Cotmore, 1988; Chen et al., 1988), although certain steps in the replication mechanism may require the synthesis of an RNA primer internally near the 5′ end of the viral strand (Rhode, 1978; Rhode & Klaassen, 1982; Faust et al., 1985). Okazaki fragments have not been detected among populations of replicating viral DNA molecules, and so bulk synthesis of nascent DNA strands is believed to occur exclusively by a continuous, strand displacement mechanism.

It is far from clear which combination of host cell DNA polymerases is involved in each stage of parvovirus DNA replication or how the virus manages to usurp the host cell DNA polymerases. An essential role for DNA polymerase α in copying parental single-stranded DNA has been suggested on the basis of the inhibition of this step in the viral replicative cycle by aphidicolin (Hardt et al., 1983; Robertson et al., 1984) and by the absence of a virion-associated DNA polymerase (Pritchard et al., 1978b). Later phases of viral DNA replication are also aphidicolin sensitive (Hardt et al., 1983), and a 3-fold increase in the levels of DNA polymerase α in infected cells has been reported (Pritchard et al., 1978a). The purified DNA polymerase α–primase complex from Ehrlich mouse cells synthesized a duplex RF DNA molecule from MVM single-stranded DNA (Faust & Rankin, 1982; Faust et al., 1984) and DNA sequences conforming to the consensus formula $3'\text{-}C_2A_{1-2}(C_{2-3}/T_2)\text{-}5'$ correlated with sites of initiation of RNA-primed DNA chains on the MVM DNA template (Faust et al., 1985). DNA polymerase γ also copied a parvovirus DNA template in vitro (Kollek & Goulian, 1981), and studies with nuclei isolated from infected cells have indicated a role for DNA polymerase α as well as γ in parvovirus DNA replication (Pritchard et al., 1981; Kollek et al., 1982; Gunther et al., 1984).

In an effort to clarify the role of host cell DNA polymerases in parvovirus DNA replication, we studied the effects of MVM infection on the properties of the cellular DNA polymerase α–primase complex. In this paper we describe a novel form of DNA polymerase α that is induced in Ehrlich mouse cells by MVM infection. This form of DNA polymerase α lacks primase activity and gradually replaces the normal DNA polymerase α–primase complex as the major form of DNA polymerase α in the infected cell.

Experimental Procedures

Materials. Nonradioactive deoxyribonucleoside 5′-triphosphates, poly(dT), and *Escherichia coli* DNA polymerase I (Klenow fragment) were from P-L Pharmacia. [^{3}H]TTP (22 Ci/mmol) and [α-^{32}P]dATP (3000 Ci/mmol) were from ICN Biomedicals. All other materials were supplied as described previously (Faust & Rankin, 1982).

Cells and Virus. Ehrlich mouse tumor cells were grown to a concentration of 8.1 × 10^9 cells/mL in Joklik's medium (Gibco) supplemented with 5% fetal bovine serum as described previously (Faust & Rankin, 1982). Infections with minute virus of mice, strain MVM(p), were performed at high multiplicity (10 plaque-forming units/cell) as described (Faust & Ward, 1979; Faust et al., 1988). Infected cells were harvested by centrifugation for 10 min at 800*g*, washed with phosphate-buffered saline, and stored at −70 °C.

Purification of DNA Polymerase α. Ion-exchange resins were prepared for use as described previously (Faust & Rankin, 1982). All steps in the purification were performed at 4 °C. Buffers A, B, D, and H were as described (Faust & Rankin, 1982). Infected cells (29-mL packed cell volume) were thawed from storage at −70 °C, suspended in 50 mL of buffer H containing 1 mM PMSF, and homogenized with 50 strokes in a glass Dounce homogenizer. An S-100 fraction (I) was recovered after centrifugation in a Beckman SW 28 swinging bucket rotor at 26 000 rpm for 2 h and fractionated by ammonium sulfate precipitation as described previously (Faust & Rankin, 1982). The 0.19 g/mL ammonium sulfate fraction (II) was suspended in 25 mL of buffer A with the aid of a Dounce homogenizer and dialyzed overnight against several changes of buffer B. The sample was diluted with 20 mL of buffer B and applied to a 28-mL DEAE-cellulose column equilibrated in buffer B. The column was developed with a 0–0.25 or 0–0.20 M NaCl gradient (140 mL) in buffer B. The sample recovered from the DEAE-cellulose column (fraction III) was dialyzed against buffer B for 5 h and applied to a phosphocellulose column (28 mL). The column was developed with a 0.15–0.50 M NaCl gradient (140 mL) in buffer B. The sample (fraction IV) was dialyzed overnight against buffer B, applied to a second DEAE-cellulose column

Table I: Purification of DNA Polymerase α–Primase from MVM-Infected Ehrlich Ascites Mouse Tumor Cells[d]

fractions	DNA polymerase		primase		DNA polymerase/primase
	total act.[a] (units × 10^{-3})	sp act. (units/mg)	total act.[b] (units × 10^{-3})	sp act. (units/mg)	
(I) postmicrosomal supernatant	11.3	7.8	3.0	2.1	3.7
(II) ammonium sulfate	5.2	10.7	1.7	3.5	3.0
(III) first DEAE-cellulose	27.9	70.5	2.5	6.2	11.3
(IV) phosphocellulose	18.3	673	2.2	78.2	8.5
(V) second DEAE-cellulose	10.2	2833	1.6	444	6.4
(VI) glycerol gradient	7.8	22664[c]	0.36	3552	21.6

[a] 1 unit = 1 nmol of deoxynucleotides incorporated into acid-insoluble activated calf thymus DNA in 1 h at 37 °C. [b] 1 unit = the amount of primase that leads to the incorporation of 1 nmol of dAMP/h by *E. coli* DNA polymerase I (Klenow fragment) with poly(dT) as a template. [c] The specific activity was calculated on the basis of densitometric analysis of stained polypeptides in SDS–polyacrylamide gels. [d] Cells were harvested 24 h postinfection.

(5 mL), and eluted with a 20-mL gradient of NaCl (0–0.25 M) in buffer B. Fraction V was dialyzed against buffer B in 10% glycerol and applied to 15–30% glycerol gradient in buffer B containing 0.1 M KCl. The gradients were centrifuged at 33 000 rpm for 44 h in a Beckman SW 40 rotor or at 26 000 rpm for 53 h in a Beckman SW 28 rotor. Samples (fraction VI) were dialyzed against buffer D and stored at −70 °C.

DNA Polymerase Assay. The DNA polymerase assay mixtures contained 40 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM DTT, 5 mM KCl, 14% glycerol (v/v), 500 μg/mL BSA, 20 μM dATP, dGTP, dCTP, and TTP, 2 μCi of [^{3}H]TTP, and 500 μg/mL activated calf thymus DNA. Aliquots (2 μL) from column or ammonium sulfate fractions were incubated at 37 °C for 30 min with 20 μL of the DNA polymerase assay mixture. The reactions were terminated by the addition of 2 mL of cold 5% trichloroacetic acid containing 10 mM $NaPP_i$. The precipitate was collected by filtration with Whatman GF/C glass fiber filters and washed in 5% trichloroacetic acid and 95% ethanol. Radioactivity was measured in a Beckman liquid scintillation counter. One unit of DNA polymerase α catalyzes the polymerization of 1 nmol of deoxynucleotide/h with activated calf thymus DNA as a template-primer.

Primase Assay. The primase assay mixture usually contained 50 mM Tris-HCl (pH 7.5), 6.25 mM $MgCl_2$, 12.5 mM DTT, 6.25 mM KCl, 18.5% glycerol (v/v), 625 μg/mL BSA, 25 μM dATP, 100 μM poly(dT) (Pharmacia), 1.0 mM ATP, and 1 μCi of [α-^{32}P]dATP. In some experiments (see Table II) the dATP concentration in the primase reaction mixture was increased to 200 μM. Aliquots (2 μL) of column and ammonium sulfate fractions were incubated at 37 °C for 1 h with 20 μL of the primase assay mixture and 0.5–1.0 unit of *E. coli* DNA polymerase I (Klenow fragment). One unit of primase activity promotes the polymerization of 1 nmol of dAMP/h on a poly(dT) template.

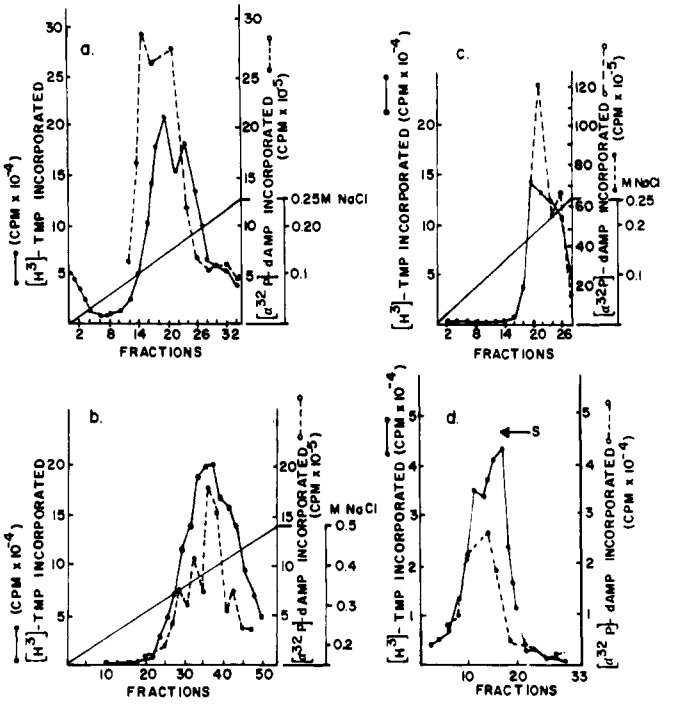

FIGURE 1: Distribution of DNA polymerase and primase activities during fractionation of extracts from MVM-infected Ehrlich mouse cells harvested 24 h postinfection: (a) first DEAE-cellulose column; (b) phosphocellulose column; (c) second DEAE-cellulose column; (d) glycerol gradient sedimentation. The DNA polymerase from the pooled fractions of the second DEAE-cellulose column was applied to a 15–30% glycerol gradient (see Experimental Procedures) and centrifuged in a Beckman SW 28 rotor at 26 000 rpm for 53 h at 4 °C. Fractions (1.0 mL) were collected from the bottom of the centrifuge tube, and a 2-μL aliquot of each fraction was assayed for DNA polymerase and primase activity ass described under Experimental Procedures. (●—●) DNA polymerase activity; (○---○) primase activity.

Results

Detection of Primase-Free DNA Polymerase α. We initially analyzed DNA polymerase α from Ehrlich mouse cells that were infected with MVM and harvested relatively early (24 h postinfection) in the MVM infectious cycle. The DNA polymerase α–primase complex was purified 3000-fold to a specific activity of 22 700 units/mg of protein (Table I). The DNA polymerase to primase ratio increased 7-fold during the purification, indicating that primase was selectively lost during purification of the DNA polymerase.

To rigorously examine the relationship between primase and DNA polymerase in the infected cell, we compared the elution profiles of these two enzymes during the preparative fractionation of cell extracts on ion-exchange columns. Two species of DNA polymerase α that eluted with 150 and 180 mM NaCl were resolved by preparative ion-exchange chromatography on a DEAE-cellulose column (Figure 1a). The DNA polymerase to primase ratio of the material in this DEAE fraction was 11.4 (Table I), approximately 4-fold greater than the value obtained for the DNA polymerase α-primase complex purified from uninfected Ehrlich mouse cells. The two DNA polymerases were pooled and applied to a phosphocellulose column. Primase and DNA polymerase eluted together during phosphocellulose column chromatography as a broad peak from 0.30 to 0.45 M NaCl (Figure 1b). When the phosphocellulose fraction was subjected to chromatography on a second DEAE-cellulose column, the DNA polymerase and primase activities once again eluted as overlapping peaks in which the trailing edge of the DNA polymerase peak exhibited relatively less primase activity, although two distinct peaks of DNA polymerase were not evident in this case due to relatively poor resolution (Figure 1c). During

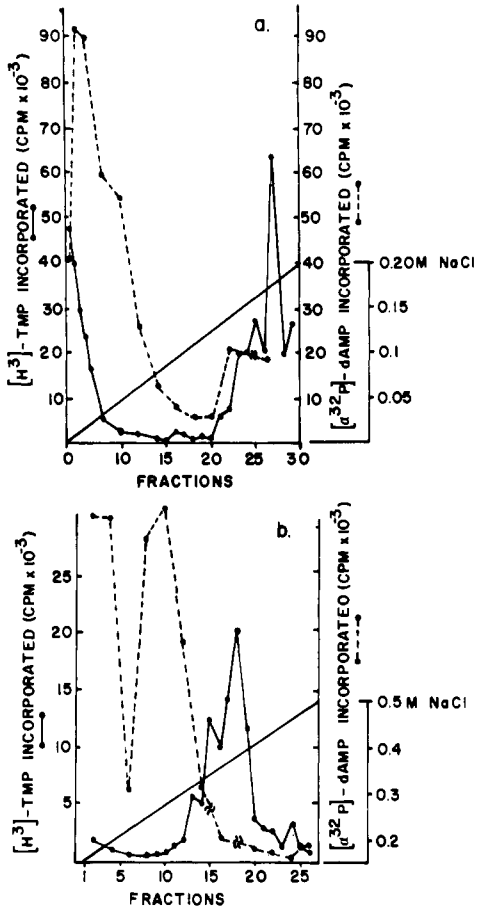

FIGURE 2: Distribution of DNA polymerase and primase activities during fractionation of extracts from MVM-infected Ehrlich mouse cells harvested 42 h postinfection: (a) first DEAE-cellulose column; (b) phosphocellulose column; (●—●) DNA polymerase activity; (O---O) primase activity.

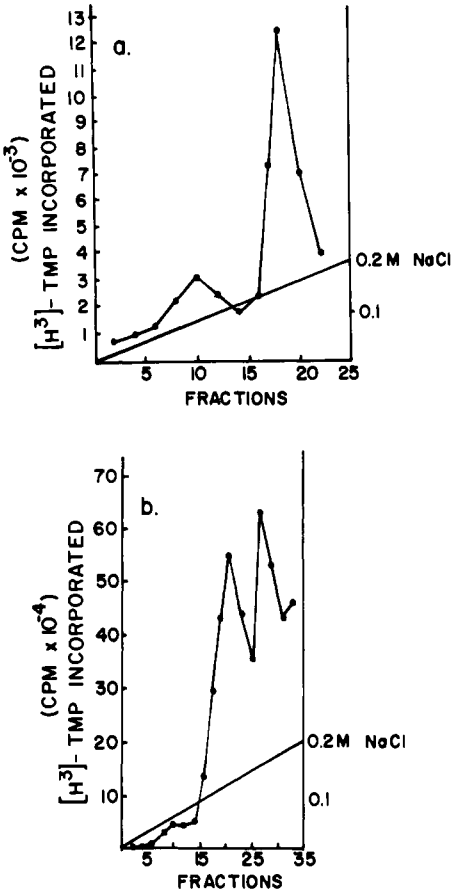

FIGURE 3: Effect of MVM infection on the chromatographic properties of DNA polymerase α from Ehrlich mouse cells. Extracts of Ehrlich cells were prepared as described under Experimental Procedures and analyzed in parallel by ion-exchange chromatography on DEAE-cellulose columns: (a) uninfected cells (first DEAE-cellulose column); (b) MVM-infected cells harvested 42 h postinfection (second DEAE-cellulose column).

preparative glycerol gradient sedimentation primase activity was associated with the leading edge of the DNA polymerase peak whereas the trailing edge of the DNA polymerase peak exhibited relatively low primase activity (Figure 1d). Two species of DNA polymerase were thus partially resolved in glycerol gradients, and their relative abundance indicated that the primase-free forms of DNA polymerase α constituted approximately 60% of the total DNA polymerase activity. The primase-free DNA polymerase sedimented more slowly than the DNA polymerase α–primase complex.

Temporal Regulation of Primase-Free DNA Polymerase α. To determine to what extent the production of primase-free forms of DNA polymerase α is temporally regulated in the viral replicative cycle, we compared the elution patterns of DNA polymerase α and primase during ion-exchange column chromatography of extracts from cells that had been infected with MVM for 42 h.

Primase bound relatively weakly to DEAE-cellulose and eluted as a major peak in 0.05 M NaCl, free of the DNA polymerase activity which eluted from DEAE-cellulose in a broad peak between 120 and 200 mM NaCl (Figure 2a). A minor peak of primase activity coeluted with the DNA polymerase at about 150 mM NaCl. The ratio of DNA polymerase to primase activity was approximately 1100, 2–3 orders of magnitude greater than the ratio determined for the highly purified DNA polymerase α–primase complex from uninfected mouse cells (Ho and Davey, unpublished data) and for DNA polymerasee α–primase purified from cells harvested 24 h after infection (Table I). Approximately 30% of the total DNA polymerase activity eluted in 20 mM NaCl and thus partially overlapped the major peak of primase activity.

The primase activity that coeluted with the DNA polymerase activity on the DEAE-cellulose column bound to phosphocellulose and was eluted as a discrete peak in 270 mM NaCl (Figure 2b). By contrast, the DNA polymerase activity eluted from phosphocellulose between 300 and 380 mM NaCl and was therefore almost completely resolved from the primase activity.

The DNA polymerase recovered from the phosphocellulose column eluted from a second DEAE-cellulose column in two peaks with 150 mM NaCl and 180 mM NaCl (Figure 3b). Thus, two major species of primase-free DNA polymerase α were present in extracts of infected cells and were reproducibly resolved by salt elution from DEAE-cellulose columns (see Figures 1a and 2a). In a parallel analysis, DNA polymerase α in extracts of uninfected cells eluted from DEAE-cellulose with a major peak at 150 mM NaCl. The elution profile included a second peak of DNA polymerase activity at 80 mM NaCl (Figure 3a). A separate peak of DNA polymerase activity eluting from DEAE-cellulose with 180 mM NaCl was not detected in extracts of uninfected cells.

In a separate series of experiments, extracts from infected cells and mock-infected cells were fractionated by ion-exchange column chromatography on DEAE-cellulose and phosphocellulose, and the amount of DNA polymerase and primase activity recovered at each stage of purification was determined (Table II). Extracts from mock-infected cells yielded 20–55% more DNA polymerase α activity than extracts from infected

Table II: Levels of DNA Polymerase α and Primase in MVM-Infected and Mock-Infected Ehrlich Mouse Cells[a]

	total act. (units)			
	mock-infected cells		MVM-infected cells[b]	
fractions	DNA polymerase α	primase	DNA polymerase α	primase
DEAE-cellulose	1121	432 (2.6)[c]	820	192 (4.3)
phosphocellulose	428	60 (7.1)	266	11 (24)

[a] The values shown were obtained by measuring the DNA polymerase α and primase activity recovered following DEAE-cellulose and phosphocellulose ion-exchange chromatography of a 0.19 g/cm^3 $(NH_4)_2SO_4$ pellet fraction derived from 5×10^9 cells. [b] Cells were harvested 43 h postinfection and 80% of the cells were viable as judged by staining with trypan blue. [c] The numbers in parentheses denote the ratio of DNA polymerase to primase activity.

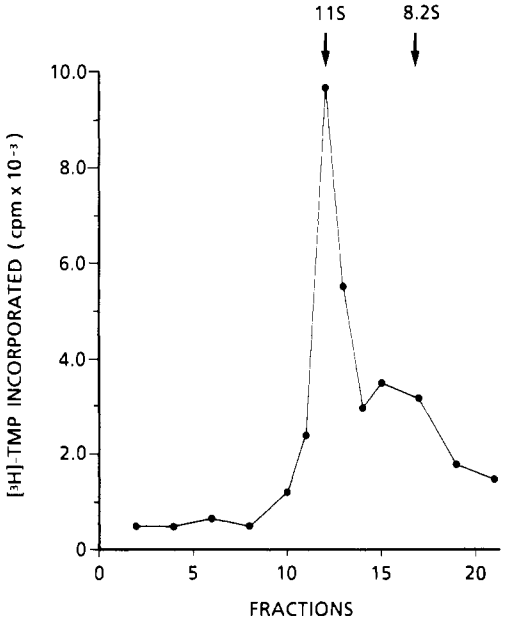

FIGURE 4: Determination of the sedimentation coefficient of primase-free DNA polymerase α from MVM-infected cells. DNA polymerase α (fraction V), purified from MVM-infected Ehrlich cells harvested 42 h postinfection, was applied to a 15–30% glycerol gradient in buffer B containing 0.1 M KCl and centrifuged in a Beckman SW 40 rotor at 33 000 rpm for 44 h at 5 °C. Portions (3 μL) of each gradient fraction were assayed for DNA polymerase activity. The standard proteins used for calibration were β-galactosidase (16 S), catalase (11.3 S), and lactate dehydrogenase (6.4 S).

cells. However, the level of primase activity associated with DNA polymerase α from infected cells was reduced 3.5-fold compared to DNA polymerase α recovered from mock-infected cells, indicating that approximately 70% of the DNA polymerase α in infected cells is free of primase activity.

Determination of the Sedimentation Coefficient. The sedimentation coefficient for primase-free DNA polymerase α was determined by comparison with β-galactosidase (16 S), catalase (11.3 S), and lactate dehydrogenase (6.4 S) as sedimentation markers. Two peaks of DNA polymerase activity were observed in glycerol gradients, one at 11 S and a second at 8.2 S (Figure 4).

Effect of Inhibitors. Aphidicolin is a potent inhibitor of DNA polymerases α and δ whereas the nucleotide polymerizing activity of DNA polymerases β and γ are unaffected by this drug (Fry & Loeb, 1986). The purine nucleotide analogues BuPdGTP and BuAdATP each inhibit DNA polymerase α at a concentration of 10^{-8} M whereas inhibition of DNA polymerase δ occurs only at much higher concentrations (Lee et al., 1985). The primase-free DNA polymerase isolated

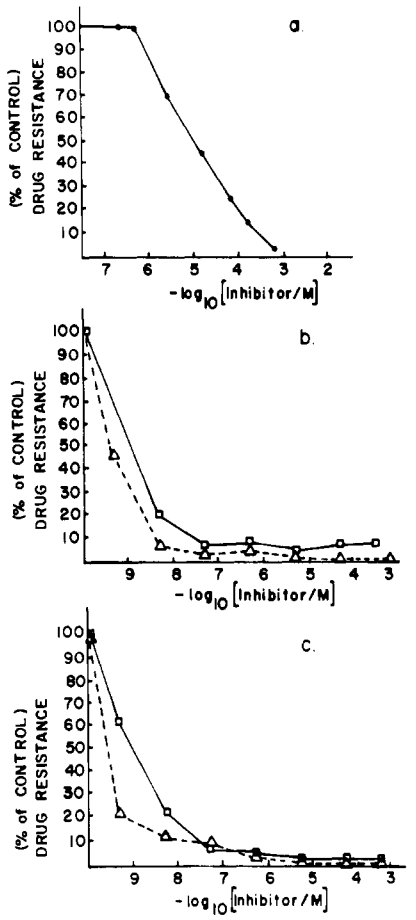

FIGURE 5: Effect of aphidicolin, BuAdATP, and BuPdGTP on the activity of primase-free DNA polymerase α. DNA polymerase (fraction V) from MVM-infected Ehrlich cells harvested 42 h postinfection was incubated at 37 °C for 60 min in the presence of increasing concentrations of (a) aphidicolin (●), (b) BuAdATP (□), or (c) BuPdGTP (□), and then assayed for the incorporation of radioactive deoxynucleotide precursor into DNA (see Experimental Procedures). The degree of drug resistance was calculated by setting the values for untreated samples at 100%. Inhibition curves obtained for DNA polymerase α (fraction V) purified from uninfected Ehrlich mouse cells are shown in panels b and c (△).

from MVM-infected mouse cells was inhibited by aphidicolin and by 10^{-8}–10^{-9} M BuPdGTP as well as BuAdATP, clearly ruling out the presence of DNA polymerase δ in our enzyme preparations (Figure 5). Primase-free DNA polymerase α from MVM-infected cells was 5–10-fold more resistant to BuAdATP than DNA polymerase α–primase from uninfected cells (Figure 5c).

Discussion

In this study we detected two primase-free forms of DNA polymerase α in extracts of MVM-infected Ehrlich mouse cells. These were present in approximately equal amounts and could be separated by sedimentation in glycerol gradients and by ion-exchange chromatography on DEAE-cellulose columns. Although far from complete, the physical and enzymological characterization of the 8.2S form of DNA polymerase α indicates that it is equivalent to the primase-free 7.3S DNA polymerase α_2 from Ehrlich mouse cells, which has a degraded DNA polymerase catalytic subunit of M_r = 78 000 (Yagura et al., 1986; Kozu et al., 1986). The two enzymes are indistinguishable in their behavior during ion-exchange chromatography on DEAE-cellulose columns, each eluting with approximately 180 mM NaCl or KCl, and both have similar, if not identical, sedimentation coefficients. The 8.2S DNA

polymerase, like the 7.3S enzyme described in the previous study by Yagura et al., probably results from degradation of a larger precursor form of DNA polymerase α. Since the 8.2S form of DNA polymerase α was not detected in uninfected Ehrlich mouse cells, MVM infection appears to cause an increase in the rate of proteolysis of higher molecular weight forms of DNA polymerase α either intracellularly or upon extraction and purification. Consistent with this suggestion, the relative abundance of the 8.2S DNA polymerase, as measured by DEAE-cellulose chromatography, increased during the infection (see Figures 1a, 2a, and 3b).

We have also detected an 11S form of DNA polymerase α that is free of primase activity. Its sedimentation rate indicates that it consists of the M_r = 182000 DNA polymerase α catalytic subunit in a tight complex with several proteins that copurify through several chromatographic steps, despite the absence of primase activity. The DNA polymerase α–primase complex from uninfected Ehrlich mouse cells has a sedimentation coefficient of 10 S and consists of four polypeptide chains of M_r = 182000, 67000, 55000, and 47000 (Faust et al., 1984, 1985). A 9S primase-free DNA polymerase α from mouse hybridoma cells consisting of two polypeptides of M_r = 185000 and 68000 has also been described (Prussak & Tseng, 1987). However, extraction of this form of the enzyme was performed in the presence of ethylene glycol and dimethyl sulfoxide, compounds that are known to cause dissociation of the normal DNA polymerase α–primase complex (Yagura et al., 1986; Pausch et al., 1988). It is therefore not clear whether the 9S DNA polymerase normally exists intracellularly. An intriguing possibility is that the 11S DNA polymerase described here is equivalent to the primase-free 9S DNA polymerase from mouse hybridoma cells and is produced because normal mechanisms of assembly of the DNA polymerase and primase subunits are disrupted during MVM infection. Alternatively, the data are consistent with the preservation of primase subunits in a complex with the DNA polymerase catalytic subunit, albeit in an inactive form. The fact that free primase was detected in extracts of infected cells (Figure 2b) is consistent with the possibility that the assembly of the DNA polymerase and primase subunits is disrupted during MVM infection.

It is of special interest to determine whether any viral proteins are associated with the primase-free 11S DNA polymerase α that we have identified in this study. The cytotoxicity of the MVM nonstructural protein NS-1 (Pintel et al., 1984; Rhode, 1987; Ozawa et al., 1988), as well as the inhibition of cellular DNA synthesis (Hardt et al., 1983), and the modification of chromatin structure (Singer & Rhode, 1978) that occur during parvovirus infection are consistent with this possibility. The M_r = 83000 NS-1 protein is covalently bound to the 5′ termini of viral DNA molecules (Chow et al., 1986; Cotmore & Tattersall, 1988; Faust et al., 1989) and has been postulated to possess a topoisomerase-like endonuclease activity (Astell et al., 1985; Chow et al., 1986; Hogan & Faust, 1986; Faust & Hogan, 1988). Although there is genetic evidence that NS-1 is essential for MVM DNA replication (Tullis et al., 1988), a specific enzymatic role for this protein remains to be defined. Unlike NS-1, the viral capsid polypeptides VP1 and VP2 are not cytotoxic (Pintel et al., 1984) and therefore are unlikely to be involved in modification of the host cell DNA polymerase α–primase complex.

The overall levels of DNA polymerase α in infected and mock-infected cells are comparable despite the fact that a major portion of the total DNA polymerase α obtained from infected cells lacks primase activity. This observation along with the fact that the infected cells were harvested at a time in the infectious cycle when at least 80% of the cells are viable and are actively synthesizing viral DNA suggests that the 11S form of DNA polymerase α, while free of primase activity, is a novel high molecular weight form of DNA polymerase α induced by virus infection as a necessary part of the viral replicative cycle. Primase-free forms of DNA polymerase α may be specifically required for the synthesis of viral DNA, a possibility that is consistent with the fact that MVM DNA replication occurs via a continuous mechanism and does not appear to involve the synthesis of RNA primers and Okazaki fragments. However, a critical role for primase in some stages of MVM DNA replication cannot be ruled out since a significant level of the DNA polymerase α–primase complex is present in infected cells.

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Registry No. DNA polymerase, 9012-90-2; primase, 64885-96-7.

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