Deoxyribonucleic Acid Polymerase from *Tetrahymena pyriformis*

PURIFICATION AND PROPERTIES OF THE MAJOR ACTIVITY IN EXPONENTIALLY GROWING CELLS

(Received for publication, July 3, 1973)

MICHAEL CRERAR AND RONALD E. PEARLMAN

From the Department of Biology, York University, Downsview, Ontario, Canada M3J 1P3

**SUMMARY**

The major DNA polymerase activity from exponentially growing *Tetrahymena pyriformis* was isolated and purified approximately 12,000-fold relative to the specific activity of DNA polymerase in crude extracts. The enzyme aggregates at low ionic strength. In solutions of ionic strength 0.25 M or greater, the enzyme exists as a single species with an approximate molecular weight of 80,000. DNA polymerase activity is associated with polymerase activity throughout all stages of purification.

In the presence of MnCl₂, the enzyme will utilize a polyribonucleotide as template in the presence of an oligodeoxynucleotide primer. The maximal velocity and $K_m$ of the enzyme are greater with dTTP than with BrdUTP as substrate when poly[d(A-T)]·poly[d(A-T)] is used as template-primer.

Ethidium bromide is a potent inhibitor of enzyme activity, but inhibition is dependent on concentration of template-primer and purity of enzyme. This suggests that care must be exercised in comparing different polymerases according to the degree of inhibition of activity by ethidium bromide. Rifamycin SV does not inhibit DNA polymerase from *Tetrahymena*, but some semisynthetic derivatives of rifamycin SV are potent inhibitors of the enzyme, the most potent being AF013.

DNA polymerase from untreated exponentially growing *Tetrahymena* appears to be similar in a number of its properties to properties reported for the DNA polymerase induced after treatment of *Tetrahymena* with ethidium bromide. However, it is not yet known whether or not the enzymes from untreated and from induced cells are the same.

DNA polymerase activity was first demonstrated in crude extracts of *Tetrahymena pyriformis* by Westergaard and Pearlman. They demonstrated that the specific activity of the DNA polymerase could be increased if the cells were treated prior to extraction of the enzyme with either ultraviolet irradiation or methotrexate plus uridine (1). Since excision-repair of the DNA from *T. pyriformis* had been found to occur after both of these treatments (9-5), it was suggested that the polymerase that was induced after these treatments might play a role in the repair process. DNA polymerase activity in crude extracts of *Tetrahymena* was separated into two peaks by Sephadex G-200 gel filtration in 0.5 M NaCl. The later eluting peak, representing a minor fraction of the total DNA polymerase activity in crude extracts of normally growing *Tetrahymena*, increased at least 35-fold in specific activity if the cells had been previously treated with either ultraviolet irradiation, electron irradiation, or methotrexate plus uridine (6). Ethidium bromide treatment of the cells also resulted in an increased specific activity of DNA polymerase in crude extracts, and it was demonstrated that this activity was associated with the mitochondrial fraction (7). These results suggested that the observed increase in specific activity of the DNA polymerase in crude extracts of treated cells was the result of production of a mitochondrial "repair" polymerase, normally present in small amounts compared to the total polymerase activity in untreated cells. This polymerase has recently been purified and characterized (8).

As one approach to studying DNA repair and replication in a eukaryotic organism, we have purified and characterized the enzyme that represents the major fraction of DNA polymerase activity in crude extracts of exponentially growing untreated *T. pyriformis*. A method for partial purification of this enzyme and a preliminary characterization of its properties have been reported (9). In this report we provide a method for fractionation of the enzyme to high purity and investigate the properties of the polymerase with regard to its utilization of various template-primers, the presence of nuclease activities, and the inhibitory effects of ethidium bromide and four rifamycin SV derivatives. Some physical properties of the enzyme are also presented. We also compare the properties of this DNA polymerase to those reported for the DNA polymerase from *Tetrahymena* induced by treatment of cells with ethidium bromide, other bacterial and eukaryotic DNA polymerases, and the viral RNA-directed DNA polymerases.
EXPERIMENTAL PROCEDURE

MATERIALS

Nucleotides and Polynucleotides—CalThymus DNA was purchased from Sigma Chemical Co. Poly(A) was obtained from Miles Laboratories Inc. and poly(dT) was prepared by the method of Biopterin. Poly(A)-oligo(dT)$_\text{15}$, poly(dA)-oligo(dT)$_\text{15}$, dATP, dCTP, dGTP, and dTTP, and poly(dA)-poly(dT)$_\text{15}$ were from P-L Biochemicals Inc. [3H]poly(dT), oligo(dT)$_\text{15}$, and poly(dA) were gifts from Dr. H. Klenow (University of Copenhagen, Copenhagen, Denmark). [3H]poly[d(A-T)]:poly[d(A-T)], or poly(dA)-poly(dT)$_\text{15}$ was prepared using the large fragment of Escherichia coli DNA polymerase I as described by Klenow and Henningham in 100 mM NaCl. DNase treatment of this compound was as described by Klenow et al. (11). [3H]dTTP (18 Ci per mmole) was obtained from New England Nuclear Corp.

Enzymes—Purified E. coli DNA polymerase I and the large fragment of this enzyme were gifts from Dr. H. Klenow (University of Copenhagen, Copenhagen, Denmark). Porcine deoxyribonuclease was from Worthington Biochemical Corp.; bovine serum albumin (crystallized and lyophilized) and ovalbumin were from Sigma Chemical Co., and human $\gamma$-globulin was from Pentex Inc.

Inhibitors—Ethidium bromide was obtained from Sigma Chemical Co. and rifamycin SV was from Calbiochem. The semisynthetic derivatives of rifamycin SV (AF/13-0-n-octyloxime of 3-formyl rifamycin SV. designated Fraction VII. 

EXPERIMENTAL PROCEDURE

MATERIALS

Organism and Medium—T. pyriformis, amiconucleate strain GL, was grown axenically in 3-liter batches in glass carboys containing 1.5% w/v proteose peptone (Difco) and 0.1% w/v liver extract fraction L (Nutritional Biochemical Co.). Cell number was determined electronically with a Coulter counter model B (Coulter Electronics Inc.). With vigorous aeration, obtained by bubbling filtered air through the medium, the doubling time was 2.5 to 3 hours. Cells, in the exponential phase of growth at a cell density of 1.5 to 2 x 10$^6$ cells per ml, were harvested, washed, and stored as previously described (9). Cells could be stored at $-20^\circ$C for 4 months without appreciable loss of DNA polymerase activity. Between 10 and 15 g wet weight of cells were obtained per 10 liters of medium.

DNA Polymerase Assay—Unless stated otherwise, reaction mixtures for measuring DNA polymerase activity with denatured calf thymus DNA as template-primer contained in 85 $\mu$l; Tris-HCl buffer, pH 7.9, 1.5 $\mu$moles; MgCl$_2$, 0.14 $\mu$moles; KC1, 20.8 $\mu$moles; dATP, dCTP, and dGTP, 0.8 $\mu$moles each; [3H]dTTP; dNTPs; bovine serum albumin (crystallized and lyophilized) and ovalbumin were from Sigma Chemical Co., and human $\gamma$-globulin was from Pentex Inc. The addition of 1G0 $\mu$l of 0.5 M KCl to 0.7 M KCl. Elution was with Buffer A + 5 M KCl. A summary of the purification procedure is given in Table I.

RESULTS

Purification and Physical Properties of the DNA Polymerase

A summary of the purification procedure is given in Table I.

Phosphocellulose Chromatography

Fraction V enzyme which was obtained by the fractionation scheme described previously (9), was chromatographed on a phosphocellulose column which had been previously equilibrated with Buffer A (20% glycerol, 10$^{-3}$ M Tris-HCl buffer, pH 7.9, 10$^{-3}$ M EDTA, and 10$^{-4}$ M diethiothreitol). The column was washed first with approximately 2 column volumes of Buffer A, and then with a similar volume of Buffer A containing 0.2 M KC1. A linear gradient, from 0.25 M KC1 to 0.7 M KC1, was applied to the column, and the polymerase activity was eluted from the column at 0.5 M KC1. Fractions containing activity were pooled, concentrated by vacuum dialysis, dialyzed against Buffer A, and designated Fraction VI.

Sephadex G-75 Chromatography

Fraction VI made up to 1 M KC1 was filtered through a column of Sephadex G-75 (superfine) which had previously been equilibrated with Buffer A + 1 M KC1. Elution was with Buffer A + 1 M KC1. DNA polymerase activity eluted at the void volume. Fractions containing DNA polymerase activity were pooled, dialyzed against Buffer A, concentrated by vacuum dialysis, and designated Fraction VII.
of the activity during a 135 month period of storage at -76°C, calf thymus DNA, or with poly(dA).oligo(dT)16, is approximately from this column was very low. Combining fractions from the column, assuming that 100% of Fraction VII protein was eluted with Fraction VIII did not stimulate polymerase activity, but even after concentration there is a loss of 40% to 50% in the fraction of A280, nm units under the polymerase peak. A poor purification and low yield in this fraction, as compared with that observed previously (9), was due to overloading the column; however, the specific activity and yield of Fraction VI were not affected when compared to other preparations of Fraction VI.

Fraction VIII protein concentration was estimated by calculating the fraction of A280 nm units under the polymerase peak (Fig. 1) with respect to the total A280 nm units eluting from the column, assuming that 100% of Fraction VII protein was eluted from the column.

Fractions VI, VII, and VIII were assayed with poly(dA).oligo(dT)16, as described under Methods. Addition of 33 ng and 300 µg of bovine serum albumin was made to the assay mixture for determining Fraction VII and Fraction VIII DNA polymerase activity, respectively.

Sephadex G-200 Column Chromatography

Fraction VII was filtered through a column of Sephadex G-200 (superfine) as described in the legend for Fig. 1. The polymerase activity eluted at the void volume. Fractions containing activity were pooled, concentrated by vacuum dialysis, and designated Fraction VIII. The percentage of recovery of polymerase activity from this column was very low. Combining fractions from the column that contained the majority of the A280 nm material with Fraction VIII did not stimulate polymerase activity, but activity measured with poly(dA).oligo(dT)16 as template-primer was increased 35-fold if assays were carried out in the presence of bovine serum albumin (3.5 mg per ml). This resulted in a 48% recovery of the polymerase activity from the column, and the specific activity was increased 3.5-fold with respect to Fraction VII.

Storage

Fraction VI activity is stable to freezing and thawing and can be stored at -76°C for at least 6 months without loss of appreciable polymerase activity. Fraction VII and Fraction VIII can also be stored at -76°C. Concentration of Fraction VIII to approximately 0.05 mg per ml is necessary for stabilization of the polymerase activity, but even after concentration there is a loss of 40% of the activity during a 1½ month period of storage at -76°C.

Table I

Purification of DNA polymerase from exponentially growing Tetrahymena pyriformis

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein</th>
<th>Activity</th>
<th>Specific Activity</th>
<th>Purification</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Crude homogenate</td>
<td>17,550</td>
<td>316</td>
<td>0.018</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>II. 100,000 x g supernatant</td>
<td>4,295</td>
<td>266</td>
<td>0.062</td>
<td>3.4</td>
<td>84.3</td>
</tr>
<tr>
<td>III. 30-70% (NH₄)₂SO₄</td>
<td>3,152</td>
<td>287</td>
<td>0.091</td>
<td>5.1</td>
<td>90.8</td>
</tr>
<tr>
<td>IV. Sephadex G-200 (0 M KCl)</td>
<td>1,401</td>
<td>442</td>
<td>0.315</td>
<td>17.5</td>
<td>140</td>
</tr>
<tr>
<td>V. Sephadex G-200 (1 M KCl)</td>
<td>394</td>
<td>127</td>
<td>0.323</td>
<td>17.9</td>
<td>40.3</td>
</tr>
<tr>
<td>VI. Phosphocellulose</td>
<td>3.0</td>
<td>78.3</td>
<td>26</td>
<td>1,444</td>
<td>25.0</td>
</tr>
<tr>
<td>VII. Sephadex G-75 (1 M KCl)</td>
<td>0.71</td>
<td>45.4</td>
<td>64</td>
<td>3,556</td>
<td>14.4</td>
</tr>
<tr>
<td>VIII. Sephadex G-200 (0 M KCl)</td>
<td>0.10</td>
<td>22.1</td>
<td>222</td>
<td>12,333</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*Crude homogenate was obtained from 180 g (wet weight) of T. pyriformis strain GL. The purification of DNA polymerase from Fraction I to Fraction V has been described previously (9). Poor purification and low yield in this fraction, as compared with that observed previously (9), was due to overloading the column; however, the specific activity and yield of Fraction VI were not affected when compared to other preparations of Fraction VI.

The high yield of polymerase activity in this fraction may be due to the loss of an inhibitor present in the previous fractions.

The presence of a divalent cation is an absolute requirement for activity of the DNA polymerase assayed in the presence or absence of KCl. The MgCl₂ optimum for DNA polymerase activity, assayed in the presence or absence of KCl, was determined using blue dextran 2000 (Pharmacia).

(Fraction VIII had been thawed and refrozen once during this period.)

Glycerol Gradient Centrifugation

Glycerol gradient centrifugation was performed to test for aggregation and disaggregation of the more highly purified polymerase fraction (see Fig. 6 and Ref. 9). Fraction VI polymerase was layered on 10 to 30% glycerol gradients and centrifuged for 15 hours as previously described. Fractions were collected through a hole in the bottom of the tube, and polymerase activity was assayed with poly[d(A-T)].poly[d(A-T)] as described under Methods. Fractions containing activity (29-37) were pooled, concentrated by vacuum dialysis, and designated Fraction VIII. The void volume (V₀) of the column was determined using blue dextran 2000 (Pharmacia).

Polyacrylamide Gel Electrophoresis

Urea polyacrylamide gel electrophoresis of Fraction VIII revealed one major band (Fig. 2). Attempts to recover DNA polymerase activity from gels, either by elution of the enzyme from normal and urea gels in Buffer A, or by elution of the enzyme from sodium dodecyl sulfate gels by the method of Weber and Kuter (15), were unsuccessful.

Catalytic Properties of DNA Polymerase Activity

DNA polymerase Fractions VI, VII, and VIII behave identically with respect to their catalytic activities.

Ionic Requirements

The presence of a divalent cation is an absolute requirement for activity of the DNA polymerase assayed in the presence or absence of KCl. The MgCl₂ optimum for DNA polymerase activity, assayed in the absence of KCl and with either heat-denatured calf thymus DNA, or with poly(dA).oligo(dT)16, is approxi-
TABLE II

Effectiveness of polyribohomopolymer and polydeoxyribohomopolymer strands as templates

<table>
<thead>
<tr>
<th>Template-primer</th>
<th>Divalent cation</th>
<th>Temperature</th>
<th>Specific activity</th>
<th>Activity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>poly(dA)-oligo(dT)$_{18}$</td>
<td>MgCl$_2$ (20 mM)</td>
<td>28</td>
<td>29.9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>MnCl$_2$ (0.9 mM)</td>
<td>22</td>
<td>8.5</td>
<td>28.8</td>
</tr>
<tr>
<td>poly(A)-oligo(dT)$_{18}$</td>
<td>MgCl$_2$ (20 mM)</td>
<td>28</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>MnCl$_2$ (0.9 mM)</td>
<td>22</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* The ratio of total nucleotide of poly(dA) or poly(A) to oligo(dT)$_{18}$ was 1:1. Concentrations of poly(dA)-oligo(dT)$_{18}$ and poly(A)-oligo(dT)$_{18}$ were 0.05 A$_{260}$ nm units per assay.

Polyribohomopolymer Strands versus Polydeoxyribohomopolymer Strands as Template

Poly(dA)-oligo(dT)$_{18}$ is a better template-primer for polymerase than poly(A)-oligo(dT)$_{18}$ when assayed with [aH]dTTP and either MgCl$_2$ or MnCl$_2$. When Mn$^{2+}$ is added as the sole cation, optimal activity on both poly(dA)-oligo(dT)$_{18}$ and poly(A)-oligo(dT)$_{18}$ is achieved at 0.9 mM MnCl$_2$. Temperature Effect with Poly(dA)-Oligo(dT)$_{18}$ or Poly(A)-Oligo(dT)$_{18}$ as Template-Primer

Optimal polymerase activity with poly(dA)-oligo(dT)$_{18}$ is achieved at 28° at 20 mM MgCl$_2$ (Table II). At 0.9 mM MnCl$_2$, at the same temperature, and with the same template-primer, activity is approximately 16-fold lower. With poly(A)-oligo(dT)$_{18}$ as template-primer, activity at 28° and 0.9 mM MnCl$_2$ is about 7-fold lower than that measured with poly(dA)-oligo(dT)$_{18}$. Since the $T_m$ of poly(A)-oligo(dT)$_{18}$ in 0.1 M NaCl is 27° (analysis by Boehringer Mannheim Corp.), the ratios of polymerase activities were measured at a lower temperature. At 22°, the activity measured with poly(dA)-oligo(dT)$_{18}$ at both 20 mM MgCl$_2$ and 0.9 mM MnCl$_2$ drops about 4- to 5-fold from that measured at 28°. However, the activity obtained with poly(A)-oligo(dT)$_{18}$ in the presence of 0.9 mM MnCl$_2$ at 22° is 1.1-fold greater than that measured at 28°. This results in almost equal activity with either poly(dA)-oligo(dT)$_{18}$ or poly(A)-oligo(dT)$_{18}$ at 22° and 0.9 mM MnCl$_2$. At 20 mM MgCl$_2$ with poly(A)-oligo(dT)$_{18}$, there is no activity at either 28° or 22°.
Fig. 4. Effect on DNA polymerase activity of varying the ratio of poly(dA) or poly(A) to oligo(dT)_15. Assays with poly(dA)-oligo(dT)_15 as template-primer were carried out as described under "Methods" except that the ratio of poly(dA) to oligo(dT)_15 was varied. The ratio of poly(dA) to oligo(dT)_15 was determined by the A_260 nm units of poly(dA) relative to the A_260 nm units of oligo(dT)_15 added to the assay. The concentration of oligo(dT)_15 (0.0138 A_260 nm unit per assay) was kept constant. Assays with poly(A)-oligo(dT)_15 as template-primer were similar to those with poly(dA)-oligo(dT)_15 except that 75.6 nmoles of MnCl_2 were substituted for MgCl_2. In this case, the reaction was run at 21° and the concentration of oligo(dT)_15 was 0.0123 A_260 nm unit per assay.

Varying the Ratio of Template to Primer

To obtain the optimal polymerizing activity of the enzyme on poly(dA)-oligo(dT)_15 and poly(A)-oligo(dT)_15, the enzyme was assayed with varying concentrations of polymer to oligomer in the presence of [3H]dTTP (Fig. 4). The ratio of polymer to oligomer was determined as the ratio of the A_260 nm units of poly(dA) or poly(A) to oligo(dT)_15. The enzyme was not active on either poly(dA), poly(A), or oligo(dT)_15 alone.

Maximal Velocity and K_m of DNA Polymerase with dTTP and BrdUTP

In Tetrahymena, the ratio of bromodeoxyuridine to thymidine incorporated into DNA in vivo varies depending on whether the incorporation of these nucleotides is into regions of DNA undergoing semiconservative replication or into regions of DNA undergoing repair replication. The ratio of BrdUMP to dUMP in DNA that has undergone semiconservative replication is 3 times greater than the ratio of BrdUMP to dUMP in DNA that has undergone repair-replication (4). If repair-replication and semiconservative replication in Tetrahymena are carried out by different enzyme systems, one explanation for the difference in the ratio of BrdUMP to dUMP is the presence of nuclease activity, active on both BrdUTP and dTTP, incorporated into DNA in vivo could be a difference in the affinities or maximal velocities of polymerases for these two nucleotides, or both. We have determined the K_m values and maximal velocities of the DNA polymerase from untreated cells for BrdUTP and dTTP. Incorporation of dTTP as a function of time with poly[d(A-T)]-poly[d(A-T)] as template-primer is slightly greater than the incorporation of BrdUMP (Fig. 5A). The maximal velocity of the reaction with BrdUTP as substrate is 1.08 x 10^{-4} units (Fig. 5B). Maximal velocity with dTTP as substrate is higher, 1.76 x 10^{-4} units. The K_m of dTTP (0.82 μM) is approximately 1.8 times greater than the K_m of BrdUTP (0.49 μM). Hence, both the K_m and maximal velocity of the polymerase with dTTP appear to be slightly higher than with BrdUTP.

Nuclease Activity

Nuclease activity, measured as the conversion of either [3H]poly[d(A-T)]-poly[d(A-T)] or [3H]poly[dT] into acid-soluble material, is present throughout the fractionation of the polymerase. Elution of nuclease activity from both the Sephadex G-75 (1 M KCl) and the final Sephadex G-200 (0 M KCl) columns coincides with the elution of the polymerase activity. Also the ratio of polymerase activity to nuclease activity remains constant in the last two steps of purification (Table III). To demonstrate further that these nuclease activities are associated with the polymerase, glycerol gradient centrifugation was performed. As noted earlier, aggregation and disaggregation of the polymerase activity is observed with centrifuging the polymerase on glycerol gradients containing 0 M KCl (Fig. 6A) and 0.5 M KCl (Fig. 6B), respectively. On both gradients the nuclease activities coincide with the polymerase activity (Fig. 6A and B). A slight variance in the ratio of the nuclease activity, assayed with [3H]poly[dT], to the polymerase activity in the light end of both gradients compared with the ratio over the majority of the gradient might be due to the presence of a small amount of nuclease activity in Fraction VI not associated with the polymerase. It appears, therefore, that nuclease activity, active on both [3H]poly[d(A-T)]-poly[d(A-T)] and [3H]poly[dT] is associated with the polymerase at the stage of purification represented by Fraction VI. Also, it appears likely that nuclease diphosphokinase activity is associated with the polymerase. Fraction VIII does not contain endonuclease activity toward double stranded DNA (φX 174 RF I) but contains a small amount of endonuclease activity towards single stranded DNA (fd viral DNA). Detailed characterization of the nuclease activities and of nucleoside diphosphokinase activity will be described elsewhere.
TABLE III

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Nuclease activity [3H]poly(dT)</th>
<th>DNA polymerase activity [3H]poly(dA-T)-poly(dA-T)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>units/ml enzyme</td>
<td>units/ml enzyme</td>
</tr>
<tr>
<td>IB</td>
<td>7.72</td>
<td>0.27</td>
</tr>
<tr>
<td>II</td>
<td>5.74</td>
<td>0.21</td>
</tr>
<tr>
<td>III</td>
<td>13.53</td>
<td>0.22</td>
</tr>
<tr>
<td>IV</td>
<td>21.2</td>
<td>3.66</td>
</tr>
<tr>
<td>V</td>
<td>3.94</td>
<td>0.47</td>
</tr>
<tr>
<td>VI</td>
<td>1.82</td>
<td>1.19</td>
</tr>
<tr>
<td>VII</td>
<td>1.27</td>
<td>3.68</td>
</tr>
<tr>
<td>VIII</td>
<td>1.56</td>
<td>4.06</td>
</tr>
</tbody>
</table>

* Nuclease activity was assayed as described under "Methods." One unit of activity is defined as 10 nmoles of acid-soluble material released in 30 min.

** Fraction IB is the 30,000 × g supernatant (centrifuged for 20 min) of the crude homogenate.

** DNA polymerase activity of Fractions I to V was assayed with denatured calf thymus DNA as described in "Methods" except that 0.2 μmole of MgCl₂ and 11.25 μmoles of KCl were added per 85 μl of reaction mix.

** DNA polymerase activity of Fractions VI to VIII was assayed with poly[d(A-T)]-poly[d(A-T)] as described under "Methods."

** Inhibitors of Polymerase Activity

Ethidium Bromide—Ethidium bromide is a potent inhibitor of the polymerase activity of Fraction VI and Fraction VIII polymerase is achieved with 8 to 9 μM ethidium bromide when the polymerase is assayed in the presence of poly[d(A-T)]-poly[d(A-T)]. Fraction VI DNA polymerase activity, assayed with denatured calf thymus DNA as template-primer, is inhibited 50% by 5 μM ethidium bromide. Varying the concentration of the template-primer affects the extent of inhibition of the polymerase activity. An increase from 35 to 350 μg of poly[d(A-T)]-poly[d(A-T)] per ml results in a decrease in inhibition of polymerase activity from 72 to 33% at 10 μM ethidium bromide. A 10-fold increase in the concentration of polymerase added to the reaction has only a slight effect on the percentage of inhibition of activity by this drug. The purity of the enzyme appears to have an effect on inhibition. When assayed with denatured calf thymus DNA, 23 μM ethidium bromide is required to inhibit 50% of Fraction II polymerase activity. With the same concentration of denatured calf thymus DNA, 50% inhibition of Fraction VI polymerase activity is achieved with 5 μM ethidium bromide. Hence, the observed inhibition of the polymerase by ethidium bromide appears to be determined in part by the concentration of the template-primer and by the state of purity of the polymerase.

Rifamycin Derivatives—A number of semisynthetic rifamycin SV derivatives inhibit to varying extents the activity of RNA-directed DNA polymerases isolated from oncaviruses (18-21). We have investigated the effect of four of these, AF/013, AF/ABDP, AF/ABP, and AF/AP on the DNA polymerase activity. AF/013 is the most potent inhibitor of Fraction VI polymerase activity, giving 50% inhibition at a concentration of 2.5 μg of AF/013 per ml (Fig. 7). AF/ABDP inhibits polymerase activity at lower concentrations than does AF/ABP. AF/AP and rifamycin SV, at concentrations as high as 120 μg per ml, do not inhibit the polymerase activity. Fraction VI polymerase activity is more sensitive to inhibition by these rifamycin SV derivatives than is Fraction III polymerase activity. Concentrations of AF/ABDP up to 60 μg per ml, and of AF/ABP up to 120 μg per ml, do not affect Fraction III polymerase activity. Sixty per cent of Fraction III polymerase activity is inhibited by 60 μg of AF/013 per ml. In agreement with Riva et al. (22), addition of bovine serum albumin to the assay decreases the amount of inhibition by AF/013.

** DISCUSSION **

We have purified a DNA polymerase from exponentially growing T. pyriformis approximately 12,000-fold. The specific ac-
in a low ionic strength buffer and it is possible that other DNA polymerase activity from polyacrylamide gels were unsuccessful. Polymerases, possibly of nuclear origin, have been isolated (26, 27). Specific inhibitors of reverse transcriptase might prove useful in the elucidation of the mechanism of replication of oncogenic RNA containing tumor viruses, in analysis of the possible role of the enzyme in cellular transformation and gene amplification and possibly as drugs for leukemia and cancer therapy. A number of semisynthetic rifamycin SV derivatives have been shown to inhibit to varying degrees not only reverse transcriptase of viral origin (18–21) but also bacterial and eukaryotic DNA-dependent RNA and DNA polymerases (18, 21, 45–51). Of the four rifamycin SV derivatives we have tested, we have found that AF/013 is the most potent inhibitor of the *Tetrahymena* polymerase. DNA polymerase activity is also inhibited by AF/ABDP and AF/AP, but to a lesser extent than by AF/013. Concentrations of AF/AP or rifamycin SV up to 120 μg per ml do not inhibit polymerase activity. Reverse transcriptases of viral origin (21) and a DNA polymerase isolated from human leukemic lymphoblasts (50) show a greater sensitivity to AF/013 than does our polymerase. The extent of inhibition by these derivatives. Fraction III polymerase activity is inhibited to a much less extent than Fraction VI polymerase activity by AF/013, AF/ABDP, and AF/ABP, but to a lesser extent than by AF/013. Concentrations of AF/AP or rifamycin SV up to 120 μg per ml do not inhibit polymerase activity. Reverse transcriptases of viral origin (21) and a DNA polymerase isolated from human leukemic lymphoblasts (50) show a greater sensitivity to AF/013 than does our polymerase. The extent of inhibition by these derivatives.
bromide of the *Tetrahymena* polymerase. *In vitro*, ethidium bromide and acriflavine have been shown to inhibit the activity of a variety of polymerases (7, 8, 26, 52–55). Meyer and Simpson (52) demonstrated that the mitochondrial DNA polymerase from rat liver was much more sensitive to ethidium bromide and acriflavine inhibition than the nuclear polymerase. We have found that ethidium bromide is a potent inhibitor of purified DNA polymerase from untreated *Tetrahymena*. The concentration of ethidium bromide necessary to inhibit 50% of the polymerase activity from highly purified fractions is similar to the concentration of ethidium bromide necessary to inhibit 50% of the polymerases from rat liver was much more sensitive to ethidium bromide and acriflavine (55).

It appears, therefore, that the degree of sensitivity to ethidium bromide cannot be used as a distinguishing factor for either the major polymerase activity which we have isolated from untreated *Tetrahymena*, the mitochondrial polymerase from untreated *Tetrahymena* or the induced DNA polymerase from cells treated with ethidium bromide. In agreement with this, the highly purified mitochondrial and nuclear DNA polymerases from yeast are inhibited to the same extent by acriflavine (55).

The DNA polymerase from untreated *Tetrahymena* appears to be very similar in a number of its properties to those reported (7, 8, 26, 52–55). Meyer and Simpson (52) demonstrated that the mitochondrial DNA polymerase from "..."
54. HIRSCHMAN, S. (1971) Science 173, 441
Deoxyribonucleic Acid Polymerase from *Tetrahymena pyriformis*:
PURIFICATION AND PROPERTIES OF THE MAJOR ACTIVITY IN
EXPONENTIALLY GROWING CELLS
Michael Crerar and Ronald E. Pearlman


Access the most updated version of this article at http://www.jbc.org/content/249/10/3123

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/249/10/3123.full.html#ref-list-1