PURIFICATION AND PROPERTIES OF DNA POLYMERASE $\gamma$ FROM RABBIT INTESTINAL EPITHELIAL CELLS

Institute of Oncology, Wawelska 15; 02-034 Warszawa, Poland

1. DNA polymerase $\gamma$ from the cytoplasmic fraction of rabbit intestinal epithelial cells has been purified 120 000-fold and was free of phosphatase and nuclease activities towards deoxyribonucleoside-5'-triphosphates and polynucleotides.

2. The enzyme exhibited maximal activity for activated DNA and poly(A)·oligo(dT)$_{12-18}$ at pH 8.5 in 0.25 and 0.15 M-KCl, respectively. $K_m$ values for dTTP with these two templates were 0.5 and 3.8 $\mu$M, respectively.

3. In contrast to DNA polymerases $\alpha$ and $\beta$, the enzyme replicated poly(A)·oligo(dT)$_{12-18}$ 10 times faster and poly(dA)·oligo(dT)$_{12-18}$ 5 times slower than activated DNA.

4. DNA polymerase $\gamma$ did not replicate poly(C)·oligo(dG)$_{12-18}$ or poly(Cm)·oligo(dT)$_{12-18}$. The reaction with poly(I) and poly(U) did not exceed 1% of that observed with poly(A).

5. The enzyme was inhibited in 60% by antiserum against DNA polymerase $\gamma$ from human lymphoblasts.

6. The nuclear fraction of rabbit intestinal epithelial cells contained DNA polymerase $\gamma$ with the same characteristics.

DNA-directed DNA polymerase $\gamma$, first described as reverse transcriptase of normal cells (Friedlander et al., 1972) is at present considered by some authors a mitochondrial DNA polymerase (Bolden et al., 1977; Bertazzoni et al., 1977; Hübischer et al., 1977). On account of the similarity between DNA polymerase $\gamma$ and viral reverse transcriptase, identification of the latter is difficult in the non-virus producing cells (Levis et al., 1974; Wu & Gallo, 1975). In this context the still not unequivocally established ability of DNA polymerase $\gamma$ to replicate ribosome templates such as poly(C), poly(Cm) and other templates easily replicated by reverse transcriptase is of considerable interest. For instance, DNA polymerase $\gamma$ purified from mouse myeloma and human lymphoblasts used poly(A) as the only ribosome template (Matsukage et al., 1975; Robert-Guroff et al., 1977), whereas the enzyme from HeLa

*This investigation was supported by grant no 1302 from the National Cancer Programme PR-6.

[335]
cells replicates also other polynucleotides, e.g. poly(C) (Gerard, 1975; Knopf et al., 1976). On these grounds Gerard (1975) has questioned suitability of poly(C)·oligo(dG)$_{12-18}$ as a specific template-primer for differentiating reverse transcriptase from cellular DNA polymerases $\alpha$, $\beta$ and $\gamma$, and proposed to use instead poly(Cm)·oligo(dG)$_{12-18}$ which, as shown by this author, is replicated exclusively by reverse transcriptase.

In the present study DNA polymerase $\gamma$ from a new source, i.e., rabbit intestinal epithelial cells, was purified, characterized biochemically and immunologically, and was tested for the ability to replicate polynucleotides. Because of the not elucidated relationship between DNA polymerase $\gamma$ and mitochondrial DNA polymerase, cell homogenate free of mitochondria was used for enzyme preparation. As earlier results showed that DNA polymerase $\gamma$ preparations from cytoplasmic and nuclear fractions have different characteristics (Spadari & Weissbach, 1974; Gerard, 1975), the enzyme was isolated from both cellular subfractions. Preliminary results of this work have been reported by Kamač et al. (1977).

MATERIALS AND METHODS

Substrates. Deoxyribonucleoside triphosphates were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). $^3$H-labelled deoxyribonucleoside triphosphates were obtained from the Radiochemical Centre (Amersham, England).

Templates and primers. Native calf thymus DNA (Sigma Chemical Co.) was activated with DNAase I according to Fensler & Loeb (1974). Oligo(dT)$_{12-18}$, oligo(dC)$_{12-18}$ and oligo(dG)$_{12-18}$ were obtained from P-L Biochemicals Inc. (Milwaukee, Wis., U.S.A.), and poly(A), poly(C), poly(I), poly(U), poly(dA), poly(dT), from Miles Laboratories (Elkhart, Ind., U.S.A.). Poly(Cm) was synthesized according to Žmudzka et al. (1969). Synthetic template-primers were prepared by mixing two complementary polynucleotides, or a polynucleotide and an oligonucleotide, at the concentrations of 200 $\mu$g/ml in 50 mM-Tris/HCl buffer (pH 7.8) containing 10 mM-NaCl, followed by heating to 80°C and annealing for 1 h at room temperature, and for 18 h at 4°C.

Chemicals. DE-23 cellulose and phosphocellulose were obtained from Whatman Co. (Maidstone, Kent, England) and hydroxyapatite, from Bio-Rad Lab. (Richmond, Calif., U.S.A.). Native DNA-cellulose was prepared according to Alberts & Herrick (1971); DNAase I was from Worthington Biochem. Co. (Freehold, N.J., U.S.A.).

Epithelial cells. Epithelial cells of rabbit small intestine were isolated by the procedure described previously (Kamač et al., 1977). For preparation of DNA polymerase $\gamma$ the cells were detached by incubation of intestinal mucosa for 40·80 min at 37°C in the buffer composed of 2.1 mM-KH$_2$PO$_4$, 1.2 mM-Na$_2$HPO$_4$, pH 7.4, 5.8 mM-KCl, 138 mM-NaCl, 1.5 mM-EDTA and 0.5 mM-mercaptoethanol. The cells were stored at $-26^\circ$C.

Isolation of DNA polymerase $\gamma$ from the cytoplasmic fraction of epithelial cells. Frozen cells (250 g) were thawed and suspended in 1 l of buffer A (1 mM-potassium phosphate, pH 6.8, 10 mM-NaCl and 1 mM-mercaptoethanol). All subsequent steps were
performed at 4°C. Cell suspension was homogenized in Dounce homogenizer (3×5 strokes, with 1 min intervals) and centrifuged at 1000 g for 10 min. The pellet was suspended in 100 ml of buffer B (1 mM-potassium phosphate, pH 6.8, 1 mM-MgCl₂, 320 mM-sucrose and 0.3% Triton X-100), homogenized (3×5 strokes) and centrifuged as above. Homogenization in buffer B and centrifugation was repeated. The resulting three 1000 g supernatants were pooled and centrifuged at 15000 g for 15 min. In the supernatant, concentrations of potassium phosphate and glycerol were adjusted to 20 mM and 20%, respectively, and the pH value to 7.5. The solution was loaded onto a 550 ml DE-cellulose column equilibrated with buffer C (20 mM-potassium phosphate, pH 7.5, 1 mM-mercaptoethanol, 20% glycerol and 0.02% Triton X-100). The column was washed with buffer D (50 mM-potassium phosphate, pH 7.5, 1 mM-mercaptoethanol, 20% glycerol and 0.02% Triton X-100) until free of the material absorbing at 280 nm. The polymerase was eluted with the gradient of 50 to 400 mM-potassium phosphate in buffer C; 17 ml fractions were collected and the DNA polymerase was determined in the assay system III (see below). To the active fractions diluted with 4 vol. of 10 mM-potassium phosphate, pH 7.5, containing 1 mM-mercaptoethanol, 150 ml of phosphocellosulose equilibrated with 100 mM-potassium phosphate, pH 7.5, and 1 mM-mercaptoethanol was added. The mixture was stirred for 4 h, the solution was decanted and the phosphocellosulose slurry was loaded onto a column containing a pad of 30 ml of phosphocellosulose. The column was washed with 100 mM-potassium phosphate, pH 7.5, and eluted with a gradient of 100 to 400 mM-potassium phosphate in buffer C. The fractions (7.5 ml) were tested for the DNA polymerase activity in systems I and III (Fig. 1a) and the fractions catalysing replication of poly(A)·oligo(dT)₁₂·₁₈ were collected, dialysed against buffer C and loaded onto a hydroxylapatite column (20 ml). The column was washed with two volumes of buffer D and eluted with 150 ml of a linear gradient of 50 to 400 mM-potassium phosphate in buffer C; the 1.8-ml fractions were collected (Fig. 1b) and assayed in system III. The pooled active fractions lost no activity on 6-month storage in liquid nitrogen. A portion of the preparations was dialysed against buffer C and loaded onto a column packed with 4 ml of native DNA-cellosulose. The column was washed with 8 vol. of buffer C and the polymerase was eluted with 100 ml of a linear gradient of 20 to 400 mM-potassium phosphate in buffer C. Fractions (1.2 ml) were tested in systems I and III (Fig. 1c). The active fractions (no. 62 to 78), stored in liquid nitrogen, lost no activity during 6 months.

Isolation of DNA polymerase γ from the nuclear fraction of epithelial cells. The 1000 g pellet obtained after separation of the cytoplasmic fraction, derived from 100 g of epithelial cells of rabbit small intestine, was suspended in 135 ml of buffer B without MgCl₂; the phosphate concentration of the solution was adjusted with 1 mM-potassium phosphate, pH 7.5, to 400 mM. The solution was sonicated with the M.S.E. sonicator, nine times for 15 s, at 1.7 V, with 15-s breaks, and centrifuged at 20 000 g for 20 min. The glycerol concentration in the supernatant was adjusted to 20%, and the solution was passed through a DE-23 cellulose column (500 ml) equilibrated with 400 mM-potassium phosphate, pH 7.5. The filtrate was dialysed against buffer C and loaded onto a second DE-23 cellulose column (250 ml). The column was washed with buffer
D until free of the material absorbing at 280 nm, and the enzyme was eluted with 800 ml of a linear gradient of 50 to 400 mm-potassium phosphate in buffer C; 10-ml fractions were assayed for the DNA polymerase activity in systems I and III (Fig. 1d). Fractions showing the activity towards poly(A)·oligo(dT)$_{12-18}$ were collected, dialysed against buffer C, and loaded onto a phosphocellulose column (1.2×20 cm). The column was washed with buffer D until free of the material absorbing at 280 nm, and eluted with 100 ml of a linear gradient of 50 to 400 mm-potassium phosphate, pH 7.5; fractions (1.8 ml) were assayed for the DNA polymerase activity in systems I and III (Fig. 1e). Fractions showing the activity towards poly(A)·oligo(dT)$_{12-18}$ were pooled, dialysed against buffer C containing 50% glycerol, and loaded onto a column packed with hydroxylapatite (1×5 cm). The column was washed with buffer D and the enzyme was eluted with 32 ml of a linear gradient of 50 to 400 mm-potassium phosphate in buffer C; fractions (0.8 ml) were assayed for the DNA polymerase activity in systems I and III (Fig. 1f). Fractions no. 27 to 33 were stored in liquid nitrogen, with no activity loss during 6 months.

Assay of DNA polymerases. The activities of DNA polymerases were assayed using as the template-primer either activated calf thymus DNA (system I and system II) or synthetic polynucleotides complexed with poly- or oligonucleotides (system III).

In system I the standard reaction mixture contained in a final volume of 50 μl: 40 mm-potassium phosphate, pH 7.2, 8 mm-MgCl$_2$, 0.8 mm-dithiothreitol (DTT), crystalline bovine serum albumin (1 mg/ml), activated DNA (0.3 mg/ml), dATP, dCTP, dGTP, $[^{3}H]$dTTP (80 c.p.m./pmol), each at the concentration of 0.1 mm, and the amount of the enzyme specified for each experiment.

In system II the reaction mixture contained in a final volume of 50 μl: 40 mm-Tris/HCl, pH 8.5, 100 mm-KCl, 8 mm-MgCl$_2$, 0.8 mm-DTT, crystalline bovine serum albumin (1 mg/ml), activated DNA (0.3 mg/ml), dATP, dCTP, dGTP each at the concentration of 0.1 mm, 10 μm-$[^{3}H]$dTTP (80 c.p.m./pmol), and the amount of the enzyme specified for each experiment.

In system III the reaction mixture contained in a final volume of 50 μl: 40 mm-Tris/HCl, pH 8.5, 40 mm-KCl, 0.4 mm-MnCl$_2$, 1.6 mm-DTT, crystalline bovine serum albumin (1 mg/ml), poly(A) (20 μg/ml), oligo(dT)$_{12-18}$ (10 μg/ml), 10 μm-$[^{3}H]$dTTP (80 c.p.m./pmol) and the amount of the enzyme specified for each experiment. If in this system as template-primer use was made of poly(A) (20 μg/ml) and poly(dT) (20 μg/ml), poly(dA) (20 μg/ml), poly(dC) (20 μg/ml), poly(dG) (20 μg/ml), poly(dC) (20 μg/ml) and oligo(dG)$_{12-18}$ (10 μg/ml), poly(C) (20 μg/ml) and oligo(dG)$_{12-18}$ (10 μg/ml), poly(C) (20 μg/ml) and oligo(dG)$_{12-18}$ (10 μg/ml), poly(dA) (10 μg/ml), then 20 μm-$[^{3}H]$dTTP (80 c.p.m./pmol), 10 μm-$[^{3}H]$dGTP (80 c.p.m./pmol), 10 μm-$[^{3}H]$dATP (80 c.p.m./pmol), the substrates. Incubation was carried out at 37°C for 30 min. Any deviations from standard conditions are indicated in the descriptions of particular experiments. The radioactive acid-precipitable material was collected and washed on Whatman no. 1 filters (diameter=2.5 cm) according to Bollum (1966). One unit of the enzymatic activity is defined as the amount of the enzyme catalysing incorporation of 1 nmol...
of $[^3H]dTTP$ into the acid-precipitable material with poly(A)·oligo(dT)$_{12-18}$ as a template-primer at 37°C during 30 min.

Immunochemical assay. DNA polymerase $\gamma$ (0.125 or 0.068 unit) from the cytoplasmic fraction was incubated with the IgG fraction of goat antiserum against the human lymphoblasts (NC 37 cell line) DNA polymerase $\gamma$ (Robert-Guroff & Gallo, 1977) (primary antibody), for 18 h at 4°C in 20 μl of the mixture containing albumin (25 mg/ml), 150 mM-KCl and 50 mM-Tris/HCl, pH 8.0. Then a 20 μl portion of the IgG fraction of the rabbit anti-goat IgG antiserum (secondary antibody) was added to precipitate the primary antibody plus any bound DNA polymerase $\gamma$. To ensure complete precipitation, the mixture was kept for 24 h at 4°C and then centrifuged for 15 min of 14,000 g. The enzyme activity remaining unbound in the supernatant was determined in 25 μl aliquots in the assay system III. The amount of the enzyme bound to the primary antibody was expressed as percentage of control activity, measured under the same conditions with non-immune IgG used instead of the primary antibody.

Protein determination. Protein was assayed according to Lowry et al. (1951) or, in the DNA polymerase preparation after the DNA-cellulose step, according to Böhlen et al. (1973).

RESULTS

Purification of DNA polymerase $\gamma$

The enzyme was purified according to Knopf et al. (1976) and Spadari & Weisbach (1974), from the cytoplasmic and the nuclear fractions of rabbit intestinal epithelial cells.

Table 1

Purification of DNA polymerase $\gamma$ from rabbit intestinal epithelial cells

Details of the purification procedure are given in Materials and Methods. The enzyme activity was determined with synthetic polynucleotides in system III. One unit of the enzyme activity is the amount of the enzyme catalysing incorporation of 1 nmol of $[^3H]dTTP$ into the acid-precipitable material at 37°C during 30 min with poly(A)·oligo(dT)$_{12-18}$ as a template-primer.

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Cytoplasmic fraction</th>
<th>Nuclear fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total protein (mg)</td>
<td>Activity (units)</td>
</tr>
<tr>
<td>Crude extract</td>
<td>6400</td>
<td>1064</td>
</tr>
<tr>
<td>DE-23 cellulose</td>
<td>2425</td>
<td>916</td>
</tr>
<tr>
<td>filtration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DE-23 cellulose</td>
<td>85</td>
<td>948</td>
</tr>
<tr>
<td>Phosphocellulose</td>
<td>3.0</td>
<td>1173</td>
</tr>
<tr>
<td>Hydroxylapatite</td>
<td>0.04</td>
<td>768</td>
</tr>
<tr>
<td>DNA-cellulose</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. DE-cellulose (d), phosphocellulose (a, e), hydroxylapatite (b, f) and DNA-cellulose (c) chromatography of DNA polymerase γ from the cytoplasmic (left) and nuclear (right) fraction of rabbit intestinal epithelial cells. For details of preparation see Materials and Methods. Samples (10 µl) were assayed under standard conditions with activated DNA (system I)(O) or (A)₉·(dT)₁₂₋₁₈ (system III)(●) as template-primer and 0.5 µM-[³H]dTTTP as a substrate.

The results of the purification procedure are summarized in Table 1. The 15 000 g supernatant of the crude cellular extract obtained from the cytoplasmic fraction was freed of nucleic acids which were adsorbed in DE-cellulose; DNA polymerase β was eluted from the column with 50 mM-potassium phosphate. The material eluted with 400 mM-potassium phosphate contained, as previously reported (Levis et al., 1974; Knopf et al., 1976; Siedlecki et al., 1976), DNA polymerases α and γ. The enzymes were initially separated by chromatography on phosphocellulose (Fig. 1a). DNA polymerase α (fractions no. 40 - 60) exhibited the activity only with the activated DNA. Fractions 65 to 85 active with poly(A)·oligo(dT)₁₂₋₁₈, representing DNA polymerase γ, were next chromatographed on hydroxylapatite (Fig. 1b) and DNA-cellulose (Fig. 1c) for further purification from polymerase α. The purification procedure applied resulted in an about 120 000-fold increase in the specific activity of DNA polymerase γ up to 19 200 units/mg protein (Table 1).
The extract of the nuclear fraction was purified from nucleic acids by preliminary filtration through DE-cellulose under conditions preventing absorption of DNA polymerases. The filtrate containing the activity of DNA polymerases was absorbed on a second DE-cellulose column, from which DNA polymerase β was washed out with 50 mM-potassium phosphate, whereas the remaining polymerases were eluted with potassium phosphate of higher concentration (Fig. 1d). Fractions (no. 3 - 28) active with poly(A)· oligo(dT)12–18, representing DNA polymerase γ, were then purified on phosphocellulose (Fig. 1e) and hydroxylapatite (Fig. 1f), finally yielding an enzyme preparation the properties of which were identical with those of analogously purified DNA polymerase γ from the cytoplasmic fraction.

In some experiments (Fig. 1b,c,f) the post-hydroxylapatite and DNA-cellulose eluates exhibited two peaks replicating poly(A)· oligo(dT)12–18, a larger and a smaller one (corresponding to 10 - 30% of the former) observed also by Spadari & Weissbach (1974) and Matsukage et al. (1975).

Fig. 2. Effect of concentration of DNA polymerase γ from cytoplasmic fraction on [3H]dTMP incorporation (a) and time-course of [3H]dTMP (●, ○) or [3H]dGMP (△) incorporation by DNA polymerase γ from cytoplasmic (b) and nuclear (c) fractions of rabbit intestinal epithelial cells. Incubation mixtures described in Materials and Methods contained activated DNA (system II) (○), poly(A)· oligo(dT)12–18 (●), or poly(C)· oligo(dG)12–18 (△) (system III) as template-primers.

Fig. 3. Effect of K⁺ on the activity of DNA polymerase γ from cytoplasmic (○) and nuclear (●) fractions of rabbit intestinal epithelial cells. Incubation mixtures described in Materials and Methods, contained as the template-primers: activated DNA (system II) (a) or poly(A)· oligo(dT)12–18 (system III) (b). Concentration of K⁺ is the sum of K⁺ in the buffer and KCl.
Evaluation of the enzyme purity. DNA polymerase \( \gamma \) activity measured with poly(A) \( \cdot \) oligo(dT)\(_{12-18} \) was proportional to the amount of the enzyme used (Fig. 2a). The replication rate of activated DNA and poly(A) \( \cdot \) oligo(dT)\(_{12-18} \) was constant, at least during 150 min of the reaction (Fig. 2b,c). The enzyme was inactive in the absence of exogenous template-primer or in the presence of primer alone (Table 3).

Table 2

\( K_m \) values for dTTP of DNA polymerase \( \gamma \) from cytoplasmic and nuclear fractions of rabbit intestinal epithelial cells

\( K_m \) values were obtained by plotting the data according to Lineweaver and Burk. The reaction mixtures were as described in Materials and Methods: system II for activated DNA and system III for (A)\(_n \) \( \cdot \) (dT)\(_{12-18} \), except that increasing dTTP concentrations were used.

<table>
<thead>
<tr>
<th>Template</th>
<th>Cytoplasmic fraction (( \mu )M)</th>
<th>Nuclear fraction (( \mu )M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated DNA</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>(A)(<em>n ) ( \cdot ) (dT)(</em>{12-18} )</td>
<td>3.8</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Replication of activated DNA required four deoxynucleoside triphosphates for the maximum yield. The reaction yields measured with \(^{[3H]}\)dGTP accounted for 72% of the yield obtained with \(^{[3H]}\)dTTP (Table 3). This indicates that the isolated enzyme was not contaminated with the dGTP-degrading phosphatase (Gerard et al., 1975). The presence of the poly(C)-hydrolysing enzyme was excluded in two kinds of experiments. In the first one in which poly(C) (0.9 A\(_{260}\) unit) was incubated with 1 unit of DNA polymerase \( \gamma \) at 37°C for 2 h at pH and salt concentration of system III, no acid-soluble fraction could be detected. In the second experiment, poly(C) \( \cdot \) oligo(dG)\(_{12-18} \) was preincubated with DNA polymerase \( \gamma \) in system III without substrates for 2 h at 37°C, then after thermal inactivation of the enzyme and supplementation of the incubation mixture with \(^{[3H]}\)dGTP and the avian myeloblastosis virus (AMV) reverse transcriptase, the template-primer activity was measured. Incorporation of the isotope into the acid-precipitable fraction was the same as with poly(C) \( \cdot \) oligo(dG)\(_{12-18} \) preincubated without DNA polymerase \( \gamma \).

Properties of DNA polymerase \( \gamma \)

Effect of salts, pH and N-ethylmaleimide. With activated DNA as the template the optimal total concentration of potassium ion derived from phosphate buffer and KCl was 250 mM (Fig. 3a). A decrease in K\(^+\) concentration caused a linear drop in the enzyme activity to 40% of the value obtained under the optimal conditions. Potassium concentration required for the optimal activity of the enzyme with the poly(A) \( \cdot \) oligo(dT)\(_{12-18} \) template was 150 mM (Fig. 3b). The increase in concentration up to 110 and 190 mM diminished the enzyme activity by 78 and 75%, respectively.
At optimum salt concentrations the activity of DNA polymerase γ isolated from both cellular and nuclear fractions was maximal at pH 8.5. At pH 7.0 the activity was lowered by half.

The DNA polymerase γ isolated from both subcellular fractions was moderately sensitive to N-ethylmaleimide at 1 mm-concentration (decrease of activity by 20 - 50%) and was inhibited in 90% at the inhibitor concentration of 10 mm.

\[ K_m \text{ value and template specificity. } K_m \text{ values for dTTP were closely similar for DNA polymerase γ preparations obtained from the cytoplasmic and nuclear fractions (Table 2). Average } K_m \text{ value for the enzyme from these two fractions with the poly(A) \cdot oligo(dT)_{12\ldots18} \text{ template, was } 3.7 \ \mu m, i.e. } \text{was about six times higher than that found with the activated DNA — } 0.6 \ \mu m. \]

**Table 3**

*Template specificity of DNA polymerase γ from cytoplasmic and nuclear fractions of rabbit intestinal epithelial cells*

The incubation mixtures were as described in Materials and Methods: system II with activated DNA and system III with synthetic templates and primers.

<table>
<thead>
<tr>
<th>Template-primer</th>
<th>(^3\text{H}-\text{labelled substrate} )</th>
<th>( K^+ \text{ concn. (nm)} )</th>
<th>Relative activity of the fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cytoplasmic</td>
<td>nuclear</td>
</tr>
<tr>
<td>Activated DNA</td>
<td>dTTP</td>
<td>150</td>
<td>100</td>
</tr>
<tr>
<td>Activated DNA</td>
<td>dGTP</td>
<td>150</td>
<td>72</td>
</tr>
<tr>
<td>((\text{A})<em>{12}\cdot(\text{dT})</em>{18})</td>
<td>dTTP</td>
<td>150</td>
<td>2000</td>
</tr>
<tr>
<td>((\text{A})<em>{12}\cdot(\text{dT})</em>{18})</td>
<td>dGTP</td>
<td>150</td>
<td>2200</td>
</tr>
<tr>
<td>((\text{dA})<em>{12}\cdot(\text{dT})</em>{18})</td>
<td>dTTP</td>
<td>150</td>
<td>20</td>
</tr>
<tr>
<td>((\text{dA})<em>{12}\cdot(\text{dT})</em>{18})</td>
<td>dGTP</td>
<td>150</td>
<td>10</td>
</tr>
<tr>
<td>((\text{C})<em>{12}\cdot(\text{dG})</em>{18})</td>
<td>dATP</td>
<td>150</td>
<td>5</td>
</tr>
<tr>
<td>((\text{C})<em>{12}\cdot(\text{dG})</em>{18})</td>
<td>dGTP</td>
<td>150</td>
<td>9</td>
</tr>
<tr>
<td>((\text{C})<em>{12}\cdot(\text{dG})</em>{18})</td>
<td>dGTP</td>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>((\text{C})<em>{12}\cdot(\text{dG})</em>{18})</td>
<td>dGTP</td>
<td>150</td>
<td>20</td>
</tr>
<tr>
<td>((\text{C})<em>{12}\cdot(\text{dG})</em>{18})</td>
<td>dGTP</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>((\text{C})<em>{12}\cdot(\text{dG})</em>{18})</td>
<td>dGTP</td>
<td>150</td>
<td>8</td>
</tr>
<tr>
<td>((\text{C})<em>{12}\cdot(\text{dG})</em>{18})</td>
<td>dGTP</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>((\text{G})<em>{12}\cdot(\text{dC})</em>{18})</td>
<td>dCTP</td>
<td>150</td>
<td>20</td>
</tr>
<tr>
<td>((\text{G})<em>{12}\cdot(\text{dC})</em>{18})</td>
<td>dCTP</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>((\text{dT})_{12}\cdot\text{dT})</td>
<td>dTTP</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>((\text{dT})_{12}\cdot\text{dT})</td>
<td>dGTP</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>((\text{dT})_{12}\cdot\text{dT})</td>
<td>dGTP</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>((\text{dT})_{12}\cdot\text{dT})</td>
<td>dTTP</td>
<td>150</td>
<td>0</td>
</tr>
</tbody>
</table>

As can be seen from Table 3, the enzyme preparations from both subcellular fractions exhibited the maximum activity with poly(A) template complexed with deoxythymidyyl primer. Replication of poly(dA) \cdot oligo(dT)_{12\ldots18} proceeded with a yield of only about 20% and 1% of the values observed with activated DNA and poly(A) as the templates, respectively. The DNA polymerase γ preparations practi-
cally did not replicate poly(C)·oligo(dG)$_{12-18}$ or poly(Cm)·oligo(dG)$_{12-18}$. The relative activity values for these templates were small and approximated the values obtained with the non-complementary substrate, dTTP. The time-course of the [$^{3}$H]dGTP incorporation (Fig. 3b,c) also indicated an artifact rather than the real enzymatic reaction found with other templates. Incorporation of the precursors, dGTP, dCTP and dATP, with the template-primers poly(C)·poly(dI), poly(I)·oligo(dC)$_{12-18}$ and poly(U)·poly(dA), respectively, did not exceed 1% of that observed with poly(A) template. The reduction of salt concentration in the reaction medium did not induce dGTP incorporation into poly(C) and poly(I), (Table 3).

**Immunological characteristics.** Interaction of the isolated DNA polymerase γ with goat antiserum against DNA polymerase γ from normal human lymphoblast (cell line NC 37) was tested in a double antibody immunoprecipitation assay (Robert-Guroff & Gallo, 1977). The amount of antiserum which bound 0.1 unit of the lymphoblast DNA polymerase γ in 90% (Robert-Guroff & Gallo, 1977), inhibited 0.125 and 0.068 unit of DNA polymerase γ from rabbit intestinal epithelial cells only by 35 and 61%, respectively. This implies tissue- and/or species-dependent differences between these two DNA polymerases γ.

**DISCUSSION**

DNA polymerase γ was isolated from small intestinal epithelial cells, in which DNA polymerases α and β were previously described (Poulson et al., 1973, 1974). Adaptation of the method of Weiser (1973) for detachment of epithelial cells from rabbit small intestine afforded a relatively large amount of homogeneous material. According to microscopic evaluation, this material contained epithelial cells of the villi and crypt cells, uncontaminated with connective tissue (Kamać et al., 1977).

Preparations of DNA polymerase γ isolated from cytoplasmic and nuclear fractions of epithelial cells have the same characteristics. They display the main properties typical of DNA polymerases γ from other sources, such as human lymphoblasts (Robert-Guroff et al., 1977), mouse myeloma (Matsukage et al., 1975) or HeLa cells (Knöpf et al., 1976), which distinguish them from DNA polymerases α and β, namely: much higher activity with poly(A)·oligo(dT)$_{12-18}$ as the template-primers than with activated DNA or poly(dA)·oligo(dT)$_{12-18}$ (Matsukage et al., 1975; Knöpf et al., 1976; Robert-Guroff et al., 1977), and, as compared with polymerases α and β, distinctly lower $K_m$ values for dTTP during DNA (Spadari & Weissbach, 1974; Siedlecki et al., 1976) or poly(A)·oligo(dT)$_{12-18}$ (Yoshido et al., 1974) replication. Moreover, the enzyme isolated from rabbit intestinal epithelium was bound by antiserum against DNA polymerase γ from human lymphoblasts, which did not cross-react with DNA polymerases α and β, and with reverse transcriptase (Robert-Guroff & Gallo, 1977).

Special attention was paid to specificity of DNA polymerase γ towards polynucleotide templates. DNA polymerase γ of HeLa cells (Spadari & Weissbach, 1974), in addition to poly(A), replicates also poly(I), poly(C) and poly(U), whereas it is inactive with poly(Cm) (Gerard, 1975). The same enzyme purified 60 000-fold
retains the ability to replicate poly(C) and poly(I) with the yield of 87% and 10%, respectively, of replication with poly(A)·oligo(dT)\textsubscript{12-18} template; in the case of poly(C), the reaction proceeds only at low salt concentration (Knopf et al., 1976). In contrast, DNA polymerase γ of intestinal epithelium, like the preparations isolated from human lymphoblasts (Robert-Guroff et al., 1977) or from mouse myeloma cells (Matsukage et al., 1975), incorporates dGTP with poly(C) template and oligo(dG)\textsubscript{12-18} or poly(dI) as a primer, or else with poly(Cm)·oligo(dG)\textsubscript{12-18} in a yield not exceeding 1% of the replication with poly(A)·oligo(dT)\textsubscript{12-18} template. It may be mentioned that for AMV reverse transcriptase poly(C)·oligo(dG)\textsubscript{12-18} and poly(Cm)·oligo(dG)\textsubscript{12-18} templates are as effective as poly(A)·oligo(dT)\textsubscript{12-18}, whereas poly(C)·poly(dI) is several times better (Spiegelman et al., 1970; Mikke et al., 1976).

The low yield of the poly(C) template replication is not due to the action of phosphatase and nuclease decomposing dGTP or poly(C). Probably it is not caused, either, by the non-homogeneity of poly(C)·oligo(dG)\textsubscript{12-18} type template-primer (Mikke & Żmudzka, 1977) because the replacement of aggregated oligo(dG)\textsubscript{12-18} primer by poly(dI) does not substantially modify the poly(C) replication. Incidentally, poly(C)·oligo(dG)\textsubscript{12-18} and poly(Cm)·oligo(dG)\textsubscript{12-18}, despite their non-homogeneous structure are effectively replicated by reverse transcriptase (Gerard et al., 1974; Mikke & Żmudzka, 1977). Taking into account the established template characteristics of DNA polymerase γ, it seems that replication of poly(C) and poly(Cm) might be a good criterion for distinguishing this enzyme from reverse transcriptase.

The use of poly(C) as a template for differentiating these two enzymes, instead of poly(Cm) as proposed by Gerard (1975), makes possible to avoid such disadvantages as the unclear dependence of template activity on concentration\textsuperscript{1}, and low replication of poly(Cm)·oligo(dG)\textsubscript{12-18} by reverse transcriptase from sources other than AMV (Gerard et al., 1974; Wu & Gallo, 1975).

The available data do not permit to explain the exceptionally high activity of DNA polymerase γ with poly(A), and only indicate that this enzyme, like reverse transcriptase, DNA polymerase I of E. coli (Loeb et al., 1973) and DNA polymerase β (Chang, 1974), does not require during replication direct interaction with the C2'-position of the template.

The authors are grateful to J. Kumač for the epithelial cell samples and to M. Robert-Guroff and R. C. Gallo, National Cancer Institute, NIH, for helpful suggestions and samples of the antisera enabling immunological assay.

REFERENCES


\textsuperscript{1} Dilution of poly(Cm) in the incubation mixture from the 30 - 60 µm concentration generally used for synthetic templates to 2 µm concentration increases the dGTP incorporation rate by 80%.


Oczyszczanie I właściwości DNA polimerazy γ z komórek nablonka jelita królika

Streszczenie

1. DNA polimeraza γ z cytoplazmatycznej frakcji komórek nablonka jelita królika oczyszczono 120 000 razy. Enzym był wolny od aktywności fosfatazowej oraz nukleotydowej wobec dezoksy-nukleozydu-5'-trójfosforanów i polinukleotydów.

2. Enzym wykazał maksymalną aktywność wobec aktywowanego DNA oraz poli(A) • oligo(dT)12-18 w pH 8.5 i odpowiednio w 0.25 i 0.15 m-KCl. Wartości \( K_m \) dTTP dla tych dwu matrycy wynosiły 0.5 i 3.8 \( \mu M \).

3. Przeciwnie niż DNA polimeraza α i β enzym replikował poli(A) • oligo(dT)12-18 10 razy szybciej, a poli(dA) • oligo(dT)12-18 5 razy wolniej niż aktywowane DNA.

4. DNA polimeraza γ nie replikowała poli(C) • oligo(dG)12-18 i poli(Cm) • oligo(dG)12-18. Reakcja z poli(I) i poli(U) nie przekraczała 1% reakcji obserwowanej z poli(A).

5. Przeciwnie niż DNA polimerazę γ z jądrowej frakcji komórek nablonka jelita królika była taką samą charakterystyką.

Received 6 February, 1979.