Synthetic Template Specificity of a Deoxyribonucleic Acid Polymerase from Regenerating Rat Liver*

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SUMMARY

The template response of a purified DNA polymerase from regenerating rat liver has been studied. This enzyme does not copy a polyribonucleotide template; however, the ribo strand of a synthetic RNA-DNA hybrid can be used as an initiator for the synthesis of the complementary deoxyribonucleotide strand. This enzyme also has a marked preference for at least partially single stranded polydeoxyribonucleotide templates. The copolymer poly[d(A-T).d(T-A)] is multiplied 4- or 8-fold.

The single stranded poly(dA) acts as an efficient template for the regenerating rat liver DNA polymerase, provided short complementary oligodeoxyribonucleotide initiators are added. Poly(dC) is used as a template without added initiator. Synthesis of the complementary polydeoxyribonucleotide continues in both cases until an amount of polymer equal to the amount of initial template is produced.

The enzyme does not copy poly(dG), either in a single stranded form in the presence of oligo(dC) or in a duplex form such as poly[d(C)-d(G)].

The specificities detected with synthetic polymers show that this DNA-dependent DNA polymerase is distinguishable from RNA-dependent DNA polymerases.

We previously reported the characterization of DNA polymerases present in normal rat liver and hepatomas, with special emphasis on their template specificities (1, 2). A DNA polymerase present in regenerating rat liver has been purified and optimal conditions (pH, Mg++, and K+ ion requirements, saturation concentrations of substrates and templates) for its action have been determined. The present paper deals with the use of synthetic templates by the partially purified DNA polymerase from regenerating rat liver in order to clarify the relationship between the enzyme and the initiator-template molecule.

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† The term initiator is used to designate the chain containing a free 3'OH, physically complexed with the template. The replication of the template starts from the 3'OH end of the initiator (3).

Similar experiments have been reported with DNA polymerase from calf thymus (4-8).

The major emphasis of the present study is a definition of criteria for the distinction between cellular and viral DNA polymerases (9, 10). These criteria are of great importance for studying cellular transformation by oncogenic RNA viruses since RNA-dependent DNA polymerase, a hybrid-dependent DNA polymerase, and a DNA-dependent DNA polymerase have been shown to be present in the virions (11). Such an approach has been recently pursued by Baltimore (12) and Spiegelman (13).

The use of synthetic polymers like poly[d(T)-(A)], poly[d(C)-(G)], or poly[A]-[U] allows extremely sensitive detection of the viral reverse transcriptase (14). Using these templates, several authors have reported the detection of a hybrid-dependent activity in normal mammalian cells (15-18). Cavalieri (19) first demonstrated that Escherichia coli DNA polymerase I can use RNA or DNA-RNA hybrids as templates. These results have been recently confirmed at least for some steps of purification of E. coli polymerase I (12, 13, 20). In contrast, two purified synthetic RNA-dependent DNA polymerases can be clearly distinguished from the DNA-dependent DNA polymerases of HeLa cells (21, 22). The purified DNA polymerase studied here is strictly DNA-dependent and has been successfully separated from a hybrid-dependent activity present in crude extracts of regenerating rat liver.

MATERIALS AND METHODS

Enzyme Preparation—Two-third partial hepatectomies were performed on 20 or 25 male WAG rats weighing 200 to 300 g by removal of the left lateral and median lobes. Forty hours after the partial hepatectomies the rats were killed by decapitation and the regenerating livers rapidly removed and placed in ice. All subsequent fractionation steps were performed at 0-4°.

Homogenates were prepared by disrupting the liver cells with a Potter homogenizer in 3 volumes of Buffer A containing 0.25 M sucrose (Buffer A:50 mM Tris-HCl, pH 7.5, 1 mM MgCl₂, 6 mM KCl, 1 mM 2-mercaptoethanol). The whole homogenate was centrifuged at 105,000 × g for 2 hours in a preparative Spinco ultracentrifuge. The postmicrosomal supernatant (Fraction 1) was separated from the sediment and from the fatty overlayer with a syringe.

The 105,000 × g supernatant fraction (350 ml) was adjusted to 30% saturation with ammonium sulfate by adding slowly
with continuous mixing 150 ml of an ammonium sulfate saturated solution of Buffer A. After 20 min, this suspension was centrifuged at 5000 rpm for 30 min. The clear supernatant layer was removed and adjusted to 40% saturation of ammonium sulfate in the same way as described above. After 20 min, the precipitate was sedimented at 5000 rpm for 30 min, and the pellet was dissolved in 50 ml of Buffer A containing 20% (w/v) glycerol. Then the solution was dialyzed against the same buffer for 18 hours (Fraction II).

Fraction II was further fractionated on a DEAE-cellulose column (DE 52 Whatman, 1.5 x 50) equilibrated with Buffer A (made 20% in glycerol) by stepwise elution. At 0.08 M NaCl, a first peak of DNA polymerase was eluted; this fraction still shows an appreciable level of activity in the absence of one to three deoxyribonucleoside triphosphates with DNA as template; moreover, the copying of the ribo strand of a poly[d(T)-d(A)] hybrid can be catalyzed by this fraction. At 0.15 M NaCl, a DNA polymerase was eluted, which requires the presence of all four deoxyribonucleoside triphosphates and a denatured DNA as template (Fraction III). At 0.25 M NaCl and 0.6 M NaCl, inactive proteins and nucleic acids were eluted, respectively.

After dialysis, Fraction III (50 to 60 ml) was adsorbed onto an hydroxylapatite column (1.5 x 25) which had been equilibrated with 0.1 M phosphate buffer pH 7.5, 6 mM KCl, 1 mM MgCl₂, 1 mM 2-mercaptoethanol and 20% glycerol (w/v). The column was washed with the same buffer and adsorbed proteins were eluted with 100 ml of a linear potassium phosphate gradient (0.1 to 0.5 M). DNA polymerase activity was eluted at 0.20 M potassium phosphate. Active fractions were pooled and dialyzed against Buffer A containing 30% glycerol (Fraction IV). At 0.25 M NaCl and 0.6 M NaCl, active protein in the preparation under the conditions of the DNA polymerase reaction assayed by the incubation of labeled DNA with the enzyme concentration and under the assay conditions used to fractionate the products of digestion according to the method of Birnboim (23). In all cases less than 2% of the initial radioactivity was eluted in the region of mononucleotides, while a defined peak was eluted in the region of undegraded DNA. Under these conditions a "nickase" activity cannot be detected and, in fact, the preincubation of denatured DNA with Fraction IV increases by 2-2.5 fold the priming activity of this DNA.

For the present study two different preparations of Fraction IV, having specific activities of 100 and 150 units per mg of protein in the presence of poly(dA-T)-d(T-A), were used. One unit of DNA polymerase is defined as the amount of activity required to convert 1 pmole of total nucleotide into an acid soluble product in 1 hour under the standard conditions specified below (Table I).

**Templates**—The double stranded copolymer poly(dA-T)-d(T-A) and single stranded homopolymers: poly(dT), poly(dC), poly(dA), poly(dG), poly(C), poly(A), poly(U), poly(G) were purchased from Miles Inc. and Biopolymers Inc. The oligodeoxyribonucleotides (dT)₁₃₋₁₅, (dG)₁₃₋₁₅, (dA)₁₃₋₁₅, and (dC)₁₃₋₁₅ were obtained from Collaborative Research Inc. Double stranded polydeoxyribonucleotides and the synthetic DNA-RNA hybrids were prepared according to Spiegelman (14). Concentrations of templates are given as concentration of mononucleotides.

**Substrates**—Deoxyribonucleoside triphosphates (dATP, dGTP, dCTP, and dTTP) and [3H]dATP were supplied by Schwartz, [α-32P]dTTP was furnished by C.E.A. (France) and [αH]dATP, [αH]dGTP, [αH]dCTP, and [αH]dTTP by Amersham Biochemical Center.

**DNA Polymerase Assay**—Unless otherwise stated in the figure legends, a standard reaction mixture of 0.25 ml including the following reagents was used throughout: 40 mM Tris-HCl (pH 7 for templates containing A or T, pH 7.6 for templates containing G or C), 3.4 mM MgCl₂, 2.4 mM KCl, 60 mM NaCl (with exception of poly(dA-T)-d(T-A) template), 200 μM dATP, dGTP, dCTP, and dTTP, and 5 μCi of [3H]-labeled or 0.25 μCi of αH-labeled deoxyribonucleoside triphosphate, and 100 μg of DNA polymerase (Fraction IV, 10 or 15 units). Incubations were carried out at 38°C, aliquots were withdrawn at various time intervals, and the acid-insoluble fraction was precipitated on Whatman GF/C glass filters in 15% perchloric acid and 2% sodium pyrophosphate. Filters were rinsed with perchloric acid, with ethanol, and then dried. The radioactivity retained on filters was measured in a Packard or Intertechnique scintillation counter. With these counters, efficiency of 3H determination was 22% and 36%, respectively.

**RESULTS**

**Detection of Hybrid-dependent Activity**—In crude extracts of regenerating rat liver an activity catalyzing the copying of the

<table>
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<th>Table I</th>
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<tr>
<td><strong>Purification of DNA polymerase from regenerating rat liver</strong></td>
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<tr>
<td>Calf thymus DNA (150 μm) was denatured by heating for 10 min at 100°C and rapid cooling at 0°C.</td>
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<tr>
<th>Fraction and step</th>
<th>Protein</th>
<th>Specific activity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Native DNA</td>
</tr>
<tr>
<td>I. 105,000 × g Supernatant</td>
<td>5500</td>
<td>0.145</td>
</tr>
<tr>
<td>II. (NH₄)₂SO₄ (30–60%)</td>
<td>705</td>
<td>1.310</td>
</tr>
<tr>
<td>III. DEAE-cellulose (peak 0.15 M NaCl)</td>
<td>115</td>
<td>3.500</td>
</tr>
<tr>
<td>IV. Hydroxylapatite</td>
<td>19</td>
<td>4.500</td>
</tr>
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</table>
Among the homopolymers poly[(dT) . (da)] and poly[(dC) . (dG)] were poor RNA-dependent DNA polymerases. D conditions: Reaction mixtures of 1 ml contained: 0.4 mM 2-mercaptoethanol, 200 μM dNTP, 0.6 μCi of [α³²P]-dUTP or 10 μCi of [³H]dGTP; 2 mM MgCl₂, 2 mM KCl, 15 mM NaCl, 2 mM MgCl₂, 2.4 mM KCl, 0.4 mM NaCl, 0.4 mM dNTP, 0.6 μCi of [α³²P]-dUTP or 10 μCi of [³H]dGTP; 10 μM template, and 0.4 ml of DNA polymerase (Fraction IV). a, poly[d(A-T)-d(T-A)]; substrates, dATP and α³²PdUTP; b, poly[(dA). (U)]; poly[(A). (U)] with [α³²P]dUTP; c, poly[(dC). (dG)]; poly[(dC). (G)] with [³H]dGTP; d, poly[(dT). (A)]; poly[(T). (A)] with [α³²P]dUTP; e, poly[(dC). (dG)]; poly[(dC). (G)] with [³H]dGTP; f, poly[(dT). (A)]; poly[(T). (A)] with [³H]dGTP. No activity was demonstrable with [³H]dGTP or [α³²P]dUTP alone.

Among the single stranded polydeoxyribonucleotides, only poly(dC) was active as a template. A very small amount of oligo(dG) present in the poly(dC) preparation could possibly act as an initiator although the purity of the poly(dC) preparation was confirmed by the following criteria: (a) spectral base properties and base analysis showed the presence of a single strand. (b) In the presence of E. coli RNA polymerase only one ribonucleoside triphosphate was polymerized, i.e. poly(dC) could stimulate the polymerization of only GTP. This control was made by Biopolymers Inc. This point will be discussed later.

Addition of short complementary oligodeoxyribonucleotides to poly(dA) or poly(dT) initiated the replication of these single stranded templates. Only an initiator that could pair with the template to form standard base pairs was active; oligo(dC) was not effective.

In all cases, the poly(dG) sequences of the polymers so far studied were not used as templates. In the presence of the double stranded homopolymer poly[(dC). (dG)] the incorporation of dGMP was considerably greater than that of dCMP. In contrast to poly(dA) or poly(dT), poly(dG) would not act as template for dCMP polymerization even in the presence of oligo(dG). This fact is evident from the exchange reaction mixture (e.g. deoxyribonucleoside diphosphates or radiolabeled products present in the [³H]dCTP preparation). Indeed, when a calf thymus DNA was used as template, the incorporation of dCMP reflected the base composition of this template.

Furthermore, the slight incorporation of the homologous nucleotide in the presence of a single stranded polymer shows that a terminal deoxynucleotidyl transferase activity (24) does not serve as a template.

Among the polydeoxyribonucleotides, the double stranded homopolymers poly[(dT). (da)] and poly[(dC). (dG)] were poor templates while the alternating poly[d(A-T)-d(T-A)] was an excellent template. Both complementary nucleotides were poly[d(A-T)-d(T-A)] hybrid or the (dC) strand of a poly[(dC). (dG)] hybrid can be detected (unpublished results). To verify whether the DNA polymerase purified from these extracts (Fraction IV) also possessed this property, we initially studied the template abilities of various synthetic polymers under two incubation conditions (a) optimal conditions previously defined for calf thymus DNA or poly[d(A-T)-d(T-A)] (D conditions) (legend Fig. 1); (b) reaction conditions used for viral RNA-dependent DNA polymerases R conditions (See Method). dATP and [α³²P]dUTP; b, poly[(dA). (U)]; poly[(A). (U)] with [α³²P]dUTP; c, poly[(dC). (dG)]; poly[(dC). (G)] with [³H]dGTP; d, poly[(dT). (A)]; poly[(T). (A)] with [³H]dGTP; e, poly[(dC). (dG)]; poly[(dC). (G)] with [³H]dGTP; f, poly[(dT). (A)]; poly[(T). (A)] with [³H]dGTP. No activity was demonstrable with [³H]dGTP or [α³²P]dUTP alone.

As shown in Fig. 2, two optimal pH values were found: pH 7 for the use of deoxyribonucleotide polymers containing A and T as a template, and pH 7.6 for the replication of poly(dC). The effect of pH on enzymatic activity detected with calf thymus DNA or poly[d(A-T)-d(T-A)] (D conditions) (legend Fig. 1); (b) reaction conditions used for viral RNA-dependent DNA polymerases R conditions (See Method). dATP and [α³²P]dUTP; b, poly[(dA). (U)]; poly[(A). (U)] with [α³²P]dUTP; c, poly[(dC). (dG)]; poly[(dC). (G)] with [³H]dGTP; d, poly[(dT). (A)]; poly[(T). (A)] with [³H]dGTP; e, poly[(dC). (dG)]; poly[(dC). (G)] with [³H]dGTP; f, poly[(dT). (A)]; poly[(T). (A)] with [³H]dGTP. No activity was demonstrable with [³H]dGTP or [α³²P]dUTP alone.

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Furthermore, the slight incorporation of the homologous nucleotide in the presence of a single stranded polymer shows that a terminal deoxynucleotidyl transferase activity (24) does
radioactivity is rendered acid-soluble during further incubation.

presence of various synthetic polymers having dC sequences
duplicated and then stopped or continued at a very low rate
polymerase reaction proceeded until the template poly(dC) was
shows that poly(dC) was the most effective template. The

Fig. 2. Influence of pH on template efficiency of various polymers. Samples were incubated for 60 min at 38° at the
pH indicated. In 40 mM Tris-HCl buffer: 1, denatured calf thymus DNA with the four deoxyribonucleoside triphosphates; 2a,
poly(d[A-T] . d[T-A]) with dATP and [3H]dTTTP; 3, poly-
[(dT) . (A)] with [3H]dATTP; 4, poly[(dT) . (T)] with [3H]dCCTP; 5,
poly (dG) with [3H]dCTTP; 6, poly(dC) with [3H]dGTP. In 50
mM potassium phosphate buffer: 2b, poly(d[A-T] . d[T-A]) with
dATP and [3H]dTTTP. Activity was expressed in nanomoles of
labeled deoxyribonucleotide incorporated by 1 ml of enzyme in 1
hour.

not account for the differences in template properties of these
polymers.

A comparison of the template capabilities of the ribo strand
and the deoxyribo strand of the heteroduplexes poly[(dT) . (A)]
and poly[(dC) . (G)] leads to the conclusion that the enzyme
specifically copies a deoxyribonucleotide template. Poly(A)
and poly(G) strands of the hybrid polymers are not replicated
by the enzyme, although they can be used as initiators. Addi-
tion of oligo(dT) to poly(A) did not stimulate the replication
of poly(A). Template Activity of Deoxyribonucleotide Polymers. Yield of
Reaction—Under the standard conditions described above, and
with concentrations of poly[d(A-T) . d(T-A)] ranging from 5 to
22 µM, more than a doubling of the template occurred. The
extensive synthesis (Fig. 3) observed in the presence of low
concentrations of template implies the utilization of the newly
synthesized product as template by the DNA polymerase or a
slippage mechanism with hairpin-like extensions from a main
chain as postulated by Kornberg (25) for E. coli DNA polymer-
ase I.

As shown in Fig. 4, dAMP and TMP were incorporated to
approximately the same extent, resulting in a total synthesis of
14 nmoles of acid-insoluble product for 5.5 nmoles of template
added. This synthesis is obtained in 3 hours, after which no
radioactivity is rendered acid-soluble during further incubation.

Comparison of the kinetics of [3H]dGMP incorporation in the
presence of various synthetic polymers having dC sequences
shows that poly(dC) was the most effective template. The
polymerase reaction proceeded until the template poly(dC) was
duplicated and then stopped or continued at a very low rate
(Fig. 4). After 10 hours the polymerase reaction is only in-
creased by the addition of template Poly(dC) was used as a
template without added initiator. Addition of oligo(dG) in-
creased the initial rate of synthesis without modifying the yield
of the reaction. A decrease of the total synthesis was noticeable
only when the ratio oligo(dG):poly(dC) exceeded 1, while dou-
bling the poly(dC) template concentration always doubled the
yield of the reaction (Figs. 4 and 5). 3

A different situation was observed in the case of poly(dA).
Poly(dA) alone is a poor template as a double stranded poly-
[(dA) . (dT)], but addition of oligo(dT) greatly stimulated the
incorporation of [3H]dTTP. As shown in Fig. 6, the rate of
synthesis increased in proportion to the amount of oligomer
added until the ratio oligo(dT):poly(dA) reached 1. Under
these conditions, replication of the template was complete and
the reaction ceases at 100%. When the oligo(dT):poly(dA)
ratio was greater than 1, the reaction stopped prematurely.
Within the range of oligo(dT) concentration studied, no appreci-
able terminal addition was observed.

3 As suggested by Baltimore (19), variations around the 100% value for the yield of the reaction can be attributed to errors in the
determination of the concentration of the templates. Other
courses of errors might be the differences in the efficiency of count-
ing of H in the form of [3H]dNTP or in acid-insoluble product
(26).

TABLE II
Template activity of single stranded or double stranded synthetic polymers for DNA polymerase from regenerating rat liver
(Fraction IV)

<table>
<thead>
<tr>
<th>Templates</th>
<th>With one nucleotide</th>
<th>With two complementary nucleotides</th>
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<tbody>
<tr>
<td></td>
<td>dAMP</td>
<td>dTMP</td>
</tr>
<tr>
<td>Synthetic polymers (22 µM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly[ d(A-T) . d(T-A)]</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Poly[ d(T) . d(A)]</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Poly[ d(T) . d(A)]</td>
<td>0.1</td>
<td>50.0</td>
</tr>
<tr>
<td>Poly[ d(T) . d(A)]</td>
<td>10.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Poly[ d(A) . d(A)]</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Poly[ d(T) . d(T)]</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Poly[ d(G) . d(G)]</td>
<td>2.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Poly[ d(C) . d(C)]</td>
<td>50.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Poly[ d(C) . d(C)]</td>
<td>4.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Poly[ d(C) . d(C)]</td>
<td>50.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Poly[ d(C) . d(C)]</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Poly[ d(G) . d(G)]</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>With the four deoxyribonucleotides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calf thymus DNA (150 µM)</td>
<td>6.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Heat-denatured</td>
<td>6.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Native</td>
<td>6.2</td>
<td>6.2</td>
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</table>
Fig. 3. Template activity of poly(d(A-T).d(T-A)]. Net synthesis of an acid-insoluble product. Reaction was performed under the conditions described under "Materials and Methods" in presence of varying concentrations of template. Substrates, dATP and [3H]dTTP.

Fig. 4. Time course of the polymerase reaction with various synthetic templates. The incubation was essentially the same as in the standard assay system, except for the total volume (1 ml). Aliquots of 25 µl were withdrawn at indicated times and assayed as described under "Materials and Methods." Each point represents the average of duplicate experiments. Activity was expressed in nanomoles of labeled deoxyribonucleotide incorporated in the presence of 100 µg of enzyme and 5.5 nmoles of each template.

The late self-initiation described by Billum (6) for calf thymus DNA polymerase was not obtained on poly(dA) with DNA polymerase from regenerating rat liver. The critical factor for the replication of this polymer appears to be the concentration of free 3'OH available to start the reaction, as demonstrated by Baltimore (12) for the copying of poly(A) by the RNA-dependent DNA polymerase of avian myeloblastosis virus. The same

Fig. 5. Poly(dC) as a template with or without oligo(dG) primer. Standard reaction mixtures of 250 µl were used containing 5.5 nmoles of poly(dC) (O), with different concentrations of oligo(dG)12:1, Δ, 0.275 nmoles; 2, ●, 1.4 nmoles; 3, X, 5.5 nmoles; 4, □, 11 nmoles. A control with 2.75 nmoles of poly(dC) was included (○).

Fig. 6. Initiator requirement for synthesis on a single stranded poly(dA). Standard reactions were set up with 5.5 nmoles of poly(dA) as template and the indicated amount of oligo(dT)12-18 (a,b,c,d,e); substrate, [3H]dTTP. No activity was demonstrable with oligo(dT)12-18 and [3H]dTTP (○).
factor does not appear to be important for poly(dC), and comparison of Figs. 5 and 6 clearly shows the difference between the two types of template.

**DISCUSSION**

Using synthetic polymers, we have shown the absolute requirement of regenerating rat liver DNA polymerase for a deoxyribonucleotide template and have defined specific properties of this enzyme. In contrast to *E. coli* DNA polymerase I, the Fraction IV of regenerating rat liver DNA polymerase did not copy a ribonucleotide template; however, this enzyme could use the ribo strand of an RNA-DNA hybrid as an initiator for the copying of the complementary deoxyribo strand. The two enzymes differ, moreover, in their sensitivity to sulfhydryl group reagents. In contrast to *E. coli* DNA polymerase I, this DNA-dependent DNA polymerase from regenerating rat liver is inhibited by *p*-chloromercuribenzoate and *N*-ethylmaleimide (27) like *E. coli* DNA polymerases II (28) and III (29).

In other respects, such as the capacity to use poly(dA-T) · d(T-A) as a template and to achieve a net synthesis with this template, the present enzyme resembles *E. coli* DNA polymerase I and the DNA polymerase recently purified from normal and regenerating rat liver (30).

The capacity to catalyze the reaction with partially single stranded DNA and the requirement for a hydrogen bonded initiator for synthesis on a single stranded template poly(dA) or poly(dT) recalls the properties of calf thymus DNA polymerase (7) and *E. coli* DNA polymerase II (28). The enzyme studied in this paper differs from the rat liver polymerase purified by Berger et al. (30) which cannot utilize denatured DNA as a template.

The high rate of synthesis on poly(dC) is also a characteristic property of the present enzyme. Spiegelman et al. (14) have previously noted that homopolymer poly(dC) is a very good template for different DNA polymerases. In our experiments, this is probably due to the partially ordered structure of poly(dC) in the incubation medium (pH4 = 7.5) (31, 32), which may be conducive to a self-initiation. We have not, however, ruled out the possibility that small amounts of oligo(dG), acting as an initiator, are present either in the enzyme preparation or in the poly(dC) preparation.

In the case of poly(dG), it is probable that this polymer has a very stable secondary structure which inhibits its action as a template for DNA polymerase (32, 33).

A study of the products synthesized in the presence of known templates is rendered possible by the duplication of these templates. The different behavior of the synthetic polymers used could be related to their physical characteristics or to biological properties specific to A-T or G-C sequences (e.g., differences in the affinity of the enzyme for these sequences, involvement of these sequences in binding, initiation or termination processes) (34). Further investigation with highly purified regenerating rat liver DNA polymerase and with natural DNA should give us better understanding of the enzymatic specificities exhibited in the presence of synthetic polymers. We are in the process of studying the *in vitro* replication of the two components of *Cancer pepsis* DNA: on the one hand poly(dA-T), a duplex DNA which is predominantly an alternating copolymer of A and T with about 3 mole% of GC base pairs, on the other hand, main component with 45 mole% of GC base pairs.

The results with homopolymers demonstrate that DNA-dependent DNA polymerase and RNA-dependent DNA polymerases are distinguishable. The use of homopolymer templates with oligodeoxyribonucleotide initiators provides an effective system for testing unknown enzymes for their template preference.

The ribo strand-dependent activity detected with poly(dT) · (A) in crude extracts prepared from regenerating rat liver is separated from the DNA-dependent activity in the course of the DEAE-cellulose chromatography (see "Materials and Methods"). This fact suggests that these activities correspond to different enzymes or to at least to different parts of a complex structure which may be concerned with the regulation of replication and transcription *in vivo*. Recently Chang and Bollum (35, 36) have found several forms of DNA polymerase in eukaryotic cells (rat liver included). The low molecular weight species may behave, notably with poly(dT) · (A), as reverse transcriptases but do not use oligoribonucleotide initiators. The high molecular weight forms are the major activity in rapidly growing cells; these forms do not copy a polyribonucleotide template but use oligoribonucleotide initiators.

Both of the molecular weight forms of DNA polymerase from eukaryotic cells are inhibited by antibody directed against the high molecular weight DNA polymerase from calf thymus gland. This observation suggests a common subunit or polypeptide sequence between various forms of DNA polymerase in the cell (37). The properties of DNA polymerase from regenerating rat liver, studied here, are similar to the properties of the high molecular weight species; in fact, this enzyme is eluted in the exclusion volume of a Sephadex G-200 column (see "Materials and Methods"). We are in the process of purifying the hybrid-dependent DNA polymerase of regenerating rat liver to compare this enzyme with the DNA-dependent DNA polymerase of the same tissue and with the reverse transcriptase of oncogetic RNA viruses.

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