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Deoxyribonucleic Acid Synthesis in Cell-free Extracts

III. CATALYTIC PROPERTIES OF DEOXYRIBONUCLEIC ACID POLYMERASE II*

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SUMMARY

DNA polymerase II requires a primer to initiate synthesis on synthetic, single stranded deoxypolynucleotides. The product of the reaction is complementary to the template and is covalently attached to the primer. The frequency of errors in incorporation is less than one nucleotide in 10^5. Repair-type synthesis occurs in the 5′ to 3′ direction and proceeds until a complete duplex structure is achieved. DNA polymerase II possesses exonuclease activity which is specific for single stranded DNA. The direction of attack is exclusively 3′ to 5′.

Enzymes that catalyze the synthesis of DNA, the DNA polymerases, have been isolated from a wide variety of organisms. The polymerases from *Escherichia coli*, T4-infected cells, and calf thymus have been studied in great detail and appear to have similar catalytic properties (1). These enzymes catalyze the incorporation of deoxyribonucleoside triphosphates in the 5′ to 3′ direction and require a free 3′-hydroxyl group to initiate synthesis (2–5). In addition to catalyzing synthesis, the enzymes from *E. coli* and T4 also possess exonucleolytic activity, hydrolyzing single stranded DNA from the 3′ terminus (4, 6). The *E. coli* enzyme also promotes hydrolysis of double stranded DNA in the 5′ to 3′ direction (7, 8). Despite the wealth of information obtained with highly purified systems, the precise physiological function of the known DNA polymerases remains unclear.

In an attempt to elucidate the role of *E. coli* DNA polymerase *in vivo*, DeLucia and Cairns (9) isolated a mutant of *E. coli* lacking normal levels of DNA polymerase (polymerase I). The availability of such a mutant (Pol A_1^-) enabled several investigators to isolate a distinct, new enzyme, DNA polymerase II (10–14). An analysis of the catalytic properties of DNA polymerase II from *E. coli* will be presented. Evidence will be presented showing that this enzyme, like *E. coli* DNA polymerase I and T4 polymerase, catalyzes template-directed synthesis of DNA in the 5′ to 3′ direction by covalent attachment of the product to the 3′-hydroxyl end of a primer. DNA polymerase II also catalyzes stepwise hydrolysis of single stranded DNA from the 3′ end, but, unlike DNA polymerase I, hydrolysis of DNA from the 5′ end is not observed.

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METHODS AND MATERIALS

Nucleotides and Polymers—α-^{32}P-Labeled deoxyribonucleoside triphosphates (4 to 8 Ci per mmole) were purchased from International Chemical and Nuclear Corp. ^{3}H-Labeled deoxyribonucleoside triphosphates (8 to 15 Ci per mmole) were purchased from Schwarz BioResearch. [γ-^{32}P]ATP was prepared according to Weiss *et al.* (15). Poly(dA) and poly(dC) were generous gifts from Dr. F. Bollum. The polymer $d(G\text{-}T)_n$ was prepared as previously described (16). The oligonucleotides $(pT)_{10}$ and $(dA\text{-}C)_4$ were prepared chemically as previously described (17). The oligonucleotide $(dG)_{12\text{-}16}$ was purchased from Collaborative Research. The DNA duplex (see Fig. 2) corresponding to the nucleotide sequence 17–50 of the yeast $tRNA^{A1a}$ was a synthetic product.[1] *E. coli* [^{3}H]DNA was isolated from strain W3110 thy$^-$ (18) grown in the presence of [^{3}H]thymine. For nuclease assays, the DNA was denatured by heating to 100° for 5 min, followed by rapid cooling. The specific activity was 190 cpm per pmole of total nucleotide.

Enzymes—Bacterial alkaline phosphatase, pancreatic DNase, micrococcal nuclease, spleen phosphodiesterase, and venom phosphodiesterase were purchased from Worthington. Polynucleotide kinase was prepared from *E. coli* infected with T4 *am*N82 according to Weiss *et al.* (19). DNA polymerase II (Fraction V, 270 units per mg) was prepared as previously described (12).

Assays—Unless otherwise stated, the standard assay for DNA polymerase II (12) was used throughout. The reaction mixture (0.3 ml) contained 20 μmoles of Tris-acetate (pH 7.4); 2 μmoles of $MgCl_2$; 1 μmole of 2-mercaptoethanol; 10 nmoles each of dCTP, dGTP, dATP and TTP; template DNA; and enzyme. One of the four dNTPs was labeled with ^{3}H or α-^{32}P and the product was measured as radioactive material insoluble in 5% trichloroacetic acid. One unit of enzyme is that which catalyzes the incorporation of 1 nmole of TTP in 5 min at 30°.

For assay of synthesis on the defined sequence DNA, the reaction mixture (25 μl) contained 2.5 μmoles of potassium phosphate buffer, pH 7.4; 0.2 μmole of $MgCl_2$; 0.3 μmole of dithiothreitol;

[1] V. Sgaramella, K. Kleppe, T. Terao, N. K. Gupta, and H. G. Khorana, manuscript in preparation.

2.5 nmoles each of dATP, dCTP, dGTP, and TTP (one of which was labeled either with ^3H or α-^{32}P); 10 pmoles of synthetic DNA; and 1.5 units of enzyme. The reaction was followed by chromatography of aliquots on DEAE-cellulose paper strips as previously described (20), or by acid insolubility.

Nuclease assays were performed in a reaction mixture (0.3 ml) containing 20 μmoles of Tris-acetate (pH 8.5); 2 μmoles of $MgCl_2$; 1 μmole of 2-mercaptoethanol; 10 nmoles of denatured *E. coli* [^3H]DNA (190 cpm per pmole); and enzyme. Incubations were for 5 min at 30°. After the incubation period, the reaction mixtures were chilled to 0° and 0.1 mg of bovine serum albumin was added. Immediately thereafter, 50 μl of 50% trichloroacetic acid were added and the mixture was allowed to stand for 5 min at 0°. The insoluble material was removed by centrifugation (5 min at 1000 × *g*), and the radioactivity in the supernatant solution was determined by adding it to 20 ml of Bray's solution (21) and counting in a liquid scintillation counter.

General—Phosphorylation and isolation of labeled oligonucleotides were as described previously (20). Isolation of labeled reaction products was by Sephadex filtration at 65° (hot column) (20). Digestion of isolated products to mononucleotides and their separation for quantitative analysis were also as previously described (20). The polynucleotide [^{32}P]pT(pT)$_9$[^3H](pT)$_{80-120}$ was a product synthesized by DNA polymerase II (see Table II) starting with poly(dA) and (pT)$_{10}$. The (pT)$_{10}$ was phosphorylated at the 5′ end with [γ-^{32}P]ATP and polynucleotide kinase and was used to prime [^3H]TMP incorporation. When the primer was extended by approximately 100 nucleotides, as judged by the ratio of ^3H:^{32}P acid-insoluble nucleotide, the reaction was terminated by the addition of EDTA (50 mM) and heated for 3 min at 100°. The product was separated from unreacted material by filtration on a hot Sephadex G-50 column (1 × 100 cm), collected, evaporated to dryness, and hydrolyzed in a mixture of formic acid and diphenylamine (22) to remove poly(dA). The radioactive product was again isolated by filtration on a hot Sephadex column as above. The specific activity of the ^{32}P was 8×10^3 cpm per pmole and the ^3H was 2×10^2 cpm per pmole.

TABLE I

Primer requirement

The complete system is described under "Methods and Materials." The template DNA (40 nmoles) was exonuclease III-treated calf thymus DNA (12). [^3H]TTP (200 cpm per pmole) was used to assay DNA synthesis with calf thymus DNA, poly(dA), (pT)$_{10}$. [^3H]dGTP (200 cpm per pmole) was used to assay synthesis with poly(dC) and (pdG)$_{12-16}$ and [^3H]dATP (200 cpm per pmole) for poly(dG-T)$_n$ and (dA-C)$_4$. In all cases 4.0 nmoles of polymer and 0.4 nmole of oligonucleotide were used. The values shown represent the acid-insoluble product obtained after 5 min of incubation with 0.07 unit of enzyme and reflect the initial rate of reaction.

Additions	Incorporation
	pmoles
Complete system	68.0
Complete system + DNA	0.8
Complete system + DNA + poly(dA)	0.9
Complete system + DNA + (pT)$_{10}$	1.0
Complete system + DNA + poly(dA) + (pdC)$_{12-16}$	1.0
Complete system + DNA + poly(dA) + (pT)$_{10}$	37.0
Complete system + DNA + poly(dC)	0.8
Complete system + DNA + (pdG)$_{12-16}$	0.6
Complete system + DNA + poly(dC) + (pT)$_{10}$	0.5
Complete system + DNA + poly(dC) + (pdG)$_{12-16}$	47.0
Complete system + DNA + poly(dG-T)$_n$	2.0
Complete system + DNA + (dA-C)$_4$	1.7
Complete system + DNA + poly(dG-T)$_n$ + (dA-C)$_4$	33.0

RESULTS

General Properties of DNA Polymerase II—For maximal activity, the enzyme requires the four deoxyribonucleoside triphosphates, Mg^{2+}, NH_4^+, a reducing agent, and exonuclease III-treated DNA (12). The enzyme is free of any endonuclease activity. Incubation of T7 [^3H]DNA (0.2 nmole) with enzyme (0.2 unit) for 30 min at 37° does not alter its sedimentation behavior at pH 13 relative to a T7 [^{32}P]DNA-untreated control. Prolonged incubation (18 hours at 37°) does not result in the release of acid-soluble radioactivity. Thus, the enzyme preparation is also free of exonuclease activity directed against native DNA.

Primer Requirement—Native or denatured DNAs are less than 5% active as templates, relative to exonuclease III-treated calf thymus DNA (12). Single stranded synthetic polymers are inactive as templates unless oligonucleotides of complementary sequence are also provided (Table I). The polymers (dC)$_n$, (dA)$_n$, and (dG-T)$_n$ were employed. The failure of any of these polymers alone to support synthesis indicates that DNA polymerase II is incapable of *de novo* chain initiation with the use of any of the four common deoxyribonucleoside triphosphates. These polymers could be rendered active as templates provided that the complementary oligonucleotides were supplied. Maximal template activity is achieved at a molar ratio of polymer to oligonucleotide of 10:1. Increasing the oligonucleotide primer to template ratio to 1:1 results in a decrease in the rate of synthesis of more than 10-fold.

The oligonucleotide requirement suggests that a duplex structure with a protruding single stranded end is required for deoxyribonucleotide incorporation.

Covalent Attachment of Product to Primer—Experiments using radioactive oligonucleotides to prime polymer-directed synthesis show that the product becomes covalently linked to the primer. The system used for this study was poly(dA) template primed with the oligonucleotide (pT)$_{10}$. The 5′ terminal phosphate of (pT)$_{10}$ was selectively labeled with ^{32}P with the use of polynucleotide kinase and [γ-^{32}P]ATP.

As shown in Table II, (pT)$_{10}$ is soluble in 5% trichloroacetic acid. As the reaction proceeds, 87% of the (pT)$_{10}$ added is rendered acid-insoluble in 20 min. Acid-insoluble product, measured as acid-insoluble ^3H, continues to accumulate with increasing time for up to 80 min. These results indicate that, on the average, each oligonucleotide primer has been extended by approximately 100 nucleotides. This is shown to be the case (see also Fig. 3).

The product of the above reaction was subjected to filtration on a hot column of Sephadex G-50 (Fig. 1, *top*). The oligonucleotide primer which prior to reaction eluted with the included material was now excluded from the gel. The positions of both the ^3H-labeled product and the 5′-^{32}P-labeled primer were coincident.

One-half of the above reaction mixture was treated with alkaline phosphatase prior to Sephadex filtration; all of the ^{32}P then eluted with the included volume at the position expected for P_i

TABLE II

Linkage of product to primer

The reaction conditions are identical with those described in Table I except poly(dA) (4.0 nmoles) was used as template and $(pT)_{10}$ (37 pmoles), phosphorylated with ^{32}P (8,000 cpm per pmole) at the 5′ terminus, was used as primer. Following incubation (0.7 unit of enzyme), aliquots (10 μl) were withdrawn, added to 0.3 ml of a solution of calf thymus DNA carrier (0.1 μmoles per ml), and the acid-insoluble radioactivity was determined. The values in the table reflect the total amount of product accumulated in a 0.3-ml reaction mixture.

Minutes	Incorporation	
	[^{3}H]TMP	[^{32}P]pT(pT)$_9$
	pmo	
0	0.8	0.3
20	1320.0	31.2
40	2100.0	31.7
80	3480.0	32.4

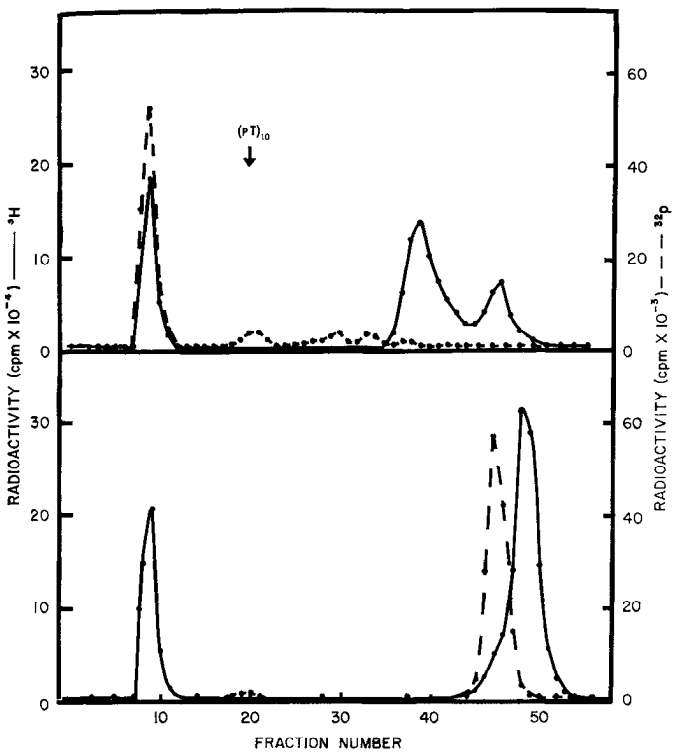

FIG. 1. Sephadex filtration of poly(dA)-directed product. The oligonucleotide $(pT)_{10}$, labeled at the 5′ terminus with ^{32}P, was used to prime poly(dA)-directed synthesis of $(pT)_n$. The details of the reaction are given in the legend to Table II. After 80 min of incubation the reaction mixture (0.3 ml) was heated to 100° for 3 min. One-half was incubated with alkaline phosphatase and the other half remained untreated. Both preparations were then adjusted to 50 mM EDTA and separately subjected to filtration on a column (1 × 110 cm) of Sephadex G-50 at 65°. The *top panel* represents the results obtained with the untreated sample and the *bottom panel* the phosphatase-treated sample. A portion of each fraction was analyzed for ^{3}H (——) and ^{32}P (- - -). The *arrow* marks the elution position of [^{32}P]pT(pT)$_9$ determined on each column prior to application of the experimental samples.

(Fig. 2, *bottom*). Thus, ^{32}P initially present on the 5′ end of the $(pT)_{10}$ primer remains susceptible to phosphomonoesterase after the reaction, indicating that addition of TMP residues must have occurred on the 3′ end of the primer.

Thus, the product of synthesis is covalently attached to the primer; the 5′ end of the primer remains intact and the primer has been extended by the addition of TMP residues to its 3′ end.

Fidelity of Synthesis—DNA polymerase II catalyzes the synthesis of a polymeric product complementary to the input template. Synthesis proceeds with an extremely high degree of accuracy.

With the polymer $(dG\text{-}T)_n$ and the primer $(dA\text{-}C)_4$, incorporation of dAMP is mutually dependent on the incorporation of dCMP and vice versa. As predicted by the sequence of the template, dAMP incorporation is equal to dCMP incorporation. The product consists of alternating of dCMP and dAMP residues (Table III).

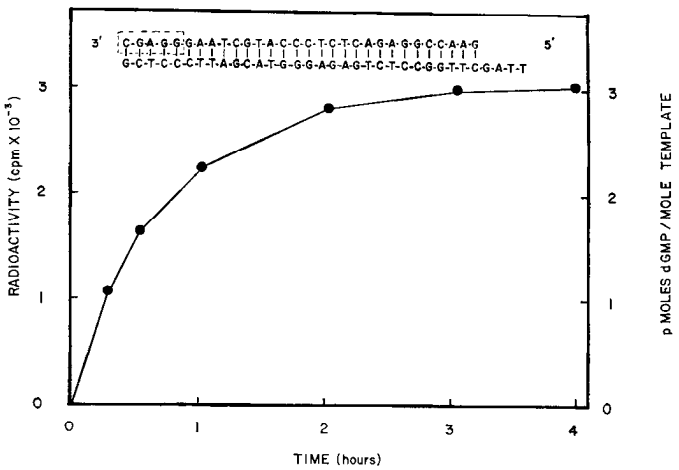

FIG. 2. "Repair" of defined sequence polymer. The polymer shown in the figure was used as the DNA substrate. The details of the reaction are given under "Methods and Materials." [α-^{32}P]-dGTP (1 × 10^3 cpm per pmole) was used to follow the accumulation of product. The product expected is delineated by a *dashed line*.

TABLE III

Nearest neighbor analysis

The reaction conditions are identical with those described in Table I. Poly(dG-T) was used as template and $(dA\text{-}C)_4$ as primer. One reaction mixture contained 1.0 nmole of [α-^{32}P]-dCTP (10^4 cpm per pmole) and 10 nmoles of each of the other unlabeled dNTPs, and the other reaction mixture contained 1.0 nmole of [α-^{32}P]dATP and 1.0 nmole of each of the other dNTPs. Following incubation for 40 min with 0.07 unit of enzyme, the reaction was terminated by the addition of EDTA (50 mM) and heated at 100° for 3 min. Each reaction mixture was then desalted, hydrolyzed with micrococcal nuclease and spleen diesterase, and the resulting 3′-mononucleotides separated by paper chromatography and the radioactivity determined. The details are given under "Methods and Materials."

Radioactive label	Radioactivity			
	Ap	Gp	Tp	Cp
	cpm			
[α-^{32}P]dCTP	2.6 × 10^6	<50	<50	<50
[α-^{32}P]dATP	<50	<50	<50	2.4 × 10^6

TABLE IV

Analysis of defined sequence product

The defined sequence polymer shown in Fig. 2 was used to direct deoxynucleotide incorporation. The expected product is delineated by the *dashed line* in Fig. 2. The details of reaction conditions and product analysis are given under "Methods and Materials." In all cases, all four dNTPs were included in the reaction mixture. The labeled nucleotides had the following specific activities: [α-^{32}P]dGTP (1×10^3 cpm per pmole); [^{3}H]-dCTP and [^{3}H]dATP (200 cpm per pmole). The values in the tables are not corrected for aliquots removed or losses during analysis.

Radioactive label	Digestion products	Radioactivity						
		dAp	pdA	dGp	Tp	dC	dCp	pdC
		cpm $\times 10^{-3}$						
[α-^{32}P]dGTP	3′-dNMP	5.1		12.0	0.14		0.13	
[α-^{32}P]dGTP	3′-dNMP	10.2		18.4	0.42		0.30	
[^{3}H]dCTP	3′-dNMP					9.8	0.10	
[^{3}H]dCTP + [^{3}H]dATP		0.94				1.1		
[^{3}H]dCTP + [^{3}H]dATP	5′-dNMP		2.1					2.0

TABLE V

Properties of DNA polymerase II-associated nuclease

Nuclease and polymerase assays were under standard conditions given under "Methods and Materials." The values in the table are the results of initial rate measurements.

Additions	Percentage of activity	
	Nuclease	Polymerase
Complete system	100	100
Complete system − Mg^{2+} + EDTA (6.7 mM)	0.5	0.5
Complete system + $(NH_4)_2SO_4$ (83 mM)	0.5	0.5
Complete system − 2-mercaptoethanol + *N*-ethylmaleimide (10 mM)	34.3	11.1
Complete system − DNA + native [^{3}H]DNA (1.2 nmoles)	0.5	

Digestion of product prepared in the presence of either [α-^{32}P]-dATP or [α-^{32}P]dCTP to 3′-mononucleotide results in the transfer of ^{32}P to the alternate nucleotide. To within an accuracy of 1 residue in 10^5, dAMP occurs adjacent to dCMP.

Direction of Synthesis—The 5′ → 3′ direction for nucleotide incorporation is further demonstrated by the use of the defined sequence synthetic duplex (Fig. 2) as a template. The sequence of the synthetic DNA duplex predicts that if synthesis were to occur in the 5′ → 3′ direction, then 3 moles of dGMP should be incorporated per mole of template. Furthermore, digestion of the reaction product to 3′-deoxymononucleotides should result in the transfer of ^{32}P to deoxyguanylate and deoxyadenylate only. This is shown to be the case (Fig. 2 and Table IV). Synthesis in the 3′ → 5′ direction does not result in dGMP incorporation.

If synthesis were to occur in both 5′ → 3′ and 3′ → 5′ directions, then the incorporation of deoxycytidylate and deoxyadenylate should be in the ratio 2:3. This has been shown (Table IV) not to occur; the ratio of dCMP:dAMP incorporation is equal. Furthermore, the products of digestion to deoxymononucleotides and deoxynucleosides show that [^{3}H]dCMP incorporation leads to [^{3}H]dC only, whereas [^{3}H]dAMP incorporation leads to deoxymononucleotide only. These results are only consistent with the direction of synthesis being uniquely 5′ → 3′. Phosphorylation of the template at its 5′ ends before synthesis by DNA polymerase II had no effect on the above results.

The results of these experiments are in accord with the predictions and are summarized in Table IV. There is no evidence for incorporation in the 3′ to 5′ direction.

Associated Exonuclease—DNA polymerase II preparations possess nuclease activity directed specifically against single stranded DNA. Nuclease digestion occurs in the 3′ to 5′ direction and the acid-soluble products are deoxymononucleotides. Under optimal conditions, the rate of nuclease digestion is one-fifth the rate of polymerization.

The general properties of the nuclease are summarized in Table V. For maximal activity the enzyme requires Mg^{2+} and a reducing agent. Activity is abolished in the presence of $(NH_4)_2SO_4$ (83 mM). Inhibition of activity in the presence of *N*-ethylmaleimide and $(NH_4)_2SO_4$ is also observed when polymerization activity is measured in the same preparation (Table V). The pH optimum is 8.5 but activity is reduced by only 20% at pH 7.5 or 9.0.

Nuclease activity is believed to be responsible for the degradation of the single stranded primer used in the experiment described in Fig. 1, *top*. ^{32}P initially present on the 5′ terminus of $(pT)_{10}$ is seen to elute at positions corresponding to shorter oligomers of thymidylic acid.

There is no apparent nuclease activity directed against native DNA. As mentioned previously, there is no detectable release of acid-soluble material following prolonged incubation of enzyme and native [^{3}H]DNA.

The nuclease activity appears to be an inherent property of the DNA polymerase II molecule. Preparation of DNA polymerase II purified to apparent homogeneity (12) has both polymerase and nuclease activities. These two activities are in a ratio of 5:1, respectively. Such a preparation was subjected to Sephadex G-200 filtration; a single peak of nuclease and polymerase activity eluted, the activities being coincident. The ratio of nuclease to polymerase activity is constant across the peak (Fig. 3).

The kinetics of heat inactivation of polymerase and nuclease activities is identical. Both activities (assayed at 30°) are unaffected by heating the enzyme preparation for 5 min at 54°. Increasing the temperature results in parallel inactivation of both activities. These results are shown in Fig. 4.

The use of a differentially labeled synthetic polymer as substrate has established the direction of nuclease attack. Digestion proceeds from the 3′ end and liberates exclusively TMP. The product of the reaction described in Table II, [^{32}P]pT$(pT)_9$-[^{3}H]$(pT)_{80-120}$, was prepared and isolated as described under "Methods and Materials." Kinetic analysis of digestion (Fig. 5) shows that approximately 100 moles of ^{3}H are rendered acid-soluble before 1 mole of ^{32}P is rendered acid-soluble. Samples taken at 5 min and 15 min were treated with EDTA to stop the reaction and subjected to paper electrophoresis. ^{3}H-Labeled material migrated as a single spot to a position coincident with a 5′-TMP marker. Thus, ^{32}P present on the 5′ end of the molecule was not found as TMP or an oligonucleotide of thymidylic acid. This result was also obtained if poly(dA) was included in the reaction mixture. It, therefore, appears that DNA polym-

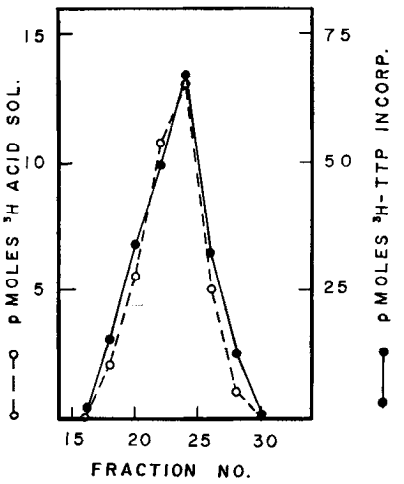

FIG. 3. Elution of polymerase and nuclease activities from Sephadex. A preparation (450 units in 2.4 ml) of apparently homogeneous DNA polymerase II (270 units per mg) was filtered through a column (2.3 × 50 cm) of Sephadex G-200; 3.0-ml fractions were collected. The details of this procedure are described elsewhere (12). Aliquots of each fraction were assayed for polymerase (●——●) and nuclease (○- - -○) activity under standard assay conditions. The substrates, [³H]TTP (200 cpm per pmole) and denatured [³H]DNA (190 cpm per pmole) were used to measure polymerase and nuclease activities respectively. Only those fractions having detectable activity are shown.

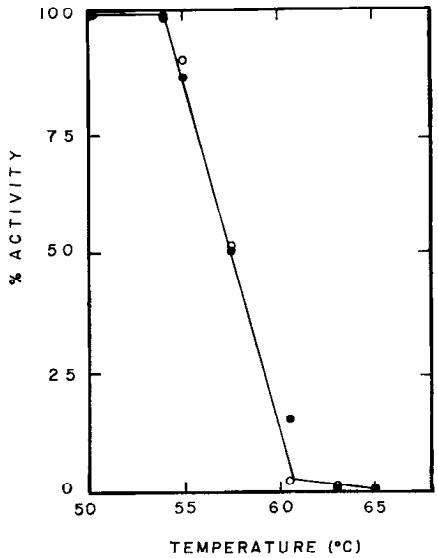

FIG. 4. Thermal inactivation of polymerase and nuclease activities. Fractions 20 to 27 (Fig. 3) were pooled and dialyzed against 21 of 0.04 potassium phosphate buffer in 50% glycerol, containing β-mercaptoethanol (5 mM), for 18 hours at 4°. Aliquots (0.1 ml) of this preparation were heated for 5 min at the indicated temperatures and then quickly cooled to 0°. The polymerase (●——●) and nuclease (○——○) activities were determined for each sample using standard assay conditions (30°). The data are plotted as per cent activity remaining relative to an unheated sample.

erase II does not have the capacity to digest DNA from the 5′ terminus.[2]

DISCUSSION

DNA polymerase II resembles the T4-induced polymerase in its catalytic properties. Template-directed synthesis proceeds with a high degree of accuracy by condensation of deoxynucleotide residues in the 5′ to 3′ direction. Chain initiation *de novo* has not been observed and synthesis occurs only by the addition of nucleotides to the 3′ end of a suitable primer. The enzyme also possesses exonucleolytic activity, hydrolyzing single stranded DNA from the 3′ terminus. No hydrolysis of DNA from the 5′ terminus has been detected.

Although we have proven that polymerase II brings about synthesis in the 5′ to 3′ direction, the possibility of polymerization in the 3′ to 5′ direction cannot be ruled out. Elongation of primers in the 3′ to 5′ direction might require a triphosphate group at the 5′ terminus (24). This possibility is currently being explored. The T4-induced polymerase has been shown not to use such a substrate for promoting synthesis in the 3′ to 5′ direction (8).

The precise physiological role of DNA polymerase II remains unclear. An assessment of this role is rendered difficult by a consideration of the complexity of the over-all process of *in vivo* DNA replication. It may be unrealistic to expect that the process of chromosome initiation and replication can be achieved *in vitro* with a single enzyme. Although the polymerase induced by T4 is clearly required for T4-specific DNA synthesis (25, 26), the enzyme fails to show, *in vitro*, properties which would appear to be necessary for the *in vivo* DNA replication. For example, no *de novo* chain initiation can be detected nor has polymerization been shown to occur in the 3′ → 5′ direction.

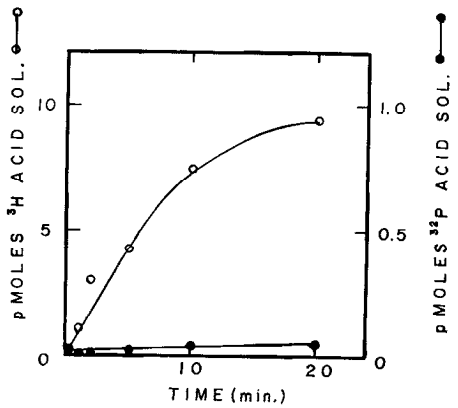

FIG. 5. Hydrolysis of differentially labeled substrate. A polymer, $(pT)_{80-120}$, labeled at the 5′ terminus with ³²P and containing 90 to 100 residues of [³H]TMP extending to the 3′ terminus was prepared as described under "Methods and Materials." The polymer (80 pmoles) was incubated with DNA polymerase II (0.09 unit) under standard assay conditions. At the times indicated aliquots were withdrawn and the acid-soluble ³H (○——○) and ³²P (●——●) were determined.

One promising approach is the analysis of mutants defective in DNA replication. A group of mutants studied previously has not shed light on this problem (27). The availability of mutants defective in DNA polymerase II will be invaluable in assigning a physiological function to this enzyme.

[2] It has been shown (D. Brutlag and A. Kornberg, unpublished observations) that the DNA polymerase II preparation described above is capable of digesting a mis-base-paired 3′-nucleotide from an otherwise duplex structure before commencing polymerization from the 3′-hydroxyl terminus. This indicates that, like DNA polymerase I and T4-induced DNA polymerase, DNA polymerase II has the capacity to edit its synthetic product (23).

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