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Exonuclease Associated with Bacteriophage T5-Induced DNA Polymerase

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T5-induced DNA polymerase has been shown to possess a 3'→5' exonucleolytic activity. The exonuclease acts on both native and denatured DNA, but the apparent rate of degradation of denatured DNA is about five times faster than that for native DNA. The enzyme appears to act only on 3'-OH ends and produces mainly 5'-dNMP's. Like polymerase activity, exonuclease activity shows a pH optimum around 8.6. Mg^{2+}, dithiothreitol, and *N*-ethylmaleimide had identical effects on both the activities. Nicked DNA was almost totally protected from exonuclease action under synthetic conditions, i.e., in the presence of 4dNTP's. Denatured DNA was partly degraded in the early phase of incubation with 4dNTP's, presumably due to unhybridized tails at the 3'-OH primer ends. However, the exonuclease activity was operative in both cases under synthetic conditions, as evidenced by template-dependent conversion of [^{3}H]dTTP to [^{3}H]dTMP.

Several bacteriophages have been shown to induce synthesis of new DNA polymerases upon infection of their respective hosts (1, 18, 25). In the case of T4 and T5 bacteriophages, it has been shown (4, 22) that the induced activity is required for phage DNA replication. Induction of a new DNA polymerase, after infection of *Escherichia coli* by bacteriophage T5, was first reported by Orr et al. (18).

The DNA polymerase induced by T4 has been shown to have both DNA polymerase and 3'→5' exonuclease activities (3, 11, 16, 17). Various authors have postulated that this exonuclease activity might be involved in correcting copying errors during replication and thus might serve a proofreader's function (2, 5, 11). Steuart et al. (21) first reported that T5 polymerase preparations had an exonuclease activity, but they did not characterize it. In this paper we present evidence from our studies with homogeneous preparations of T5 DNA polymerase which indicates that the exonuclease activity is associated with the protein having polymerase activity.

The mode of action of the exonuclease was found to be 3'→5', with a specificity toward the 3'-OH end. Internal sites (i.e., nicks in duplex DNA with 3'-OH) were also the sites of action of exonuclease. Under synthetic conditions, nicked DNA template was found to be totally protected from exonuclease action. Denatured DNA, when used as template, was partly degraded by the enzyme during the initial phase of incubation with 4dNTP's. The newly synthesized strand was degraded during synthesis, as shown by the template-dependent conversion of dNTP to dNMP.

MATERIALS AND METHODS

Organisms. Bacteriophage T5$^+$ was kindly provided by Y. Lanni. The host bacterial strain *E. coli* R15 *polA*$^-$ was received from S. Kondo (14).

Precursors and enzymes. All 4dNTP's were purchased from P-L Biochemicals. [^{3}H]dTTP was obtained from Schwarz/Mann. γ-[^{32}P]ATP was from New England Nuclear Corp. Pancreatic DNase (DNase I), micrococcal nuclease, and alkaline phosphatase were from Worthington Biochemicals Corp. [^{32}P]5'-dTMP was prepared by a method described by Fujimura (8). T4-induced polynucleotide kinase was from Miles Laboratories.

DNA preparations. Bacteriophage T7 DNA labeled with ^{32}P, which was used for most of the experiments with purified polymerase, was prepared by a procedure described by Fujimura and Roop (10). ^{32}P-labeled 5'-T7 DNA (native) and ^{32}P-labeled 5'-denatured T7 DNA were prepared by the procedure of Richardson (20).

PEI-cellulose plates. PEI-cellulose plates were purchased from Brinkmann Instruments Ltd. (MN Polygram Cel-300 PEI).

Purification of T5$^+$-induced DNA polymerase. T5$^+$-induced DNA polymerase was purified by the method of Fujimura and Roop (10). This procedure consistently yielded polymerase preparations that showed a single band on both native and sodium dodecyl sulfate-gel electrophoresis.

Conditions for polymerase assay. Each 0.3 ml of the reaction mixture for polymerase assay contained

the following: 33 μM each dATP, dTTP, dGTP, and dCTP; 500 nCi of [^{3}H]dTTP; 67 mM Tris-hydrochloride (pH 8.6); 6.7 mM $MgCl_2$; 17 mM dithiothreitol (DTT); approximately 32 nmol of ^{32}P-labeled T7 DNA (nucleotide P basis; specific activity, ~1,500 cpm/nmol); and variable amounts of enzyme. Wherever appropriate, 0.1 or 0.2 ml of reaction mixture was used. The enzyme was diluted in 0.02 M Tris-hydrochloride (pH 8.6) containing 0.1% bovine serum albumin, 2 mM DTT, and 20% glycerol (21). After the desired time of incubation at 37°C, the reaction was terminated by the addition of 2 ml of ice-cold trichloroacetic acid containing 0.01 M $Na_4P_2O_7$. The acid-insoluble material was collected on a Whatman glass-fiber filter (GF/c), washed once with 2 ml of 5% trichloroacetic acid described above and three times with 5 ml of 0.01 N HCl, and dried. The radioactivity was determined by counting in a toluene scintillator [4 g of 2,5-bis-2(5-*tert* butylbenzoxazolyl)thiophene per liter of toluene].

Exonuclease assay. Exonuclease was assayed under conditions mentioned above, except that 4dNTP's were omitted and DNA concentration varied from experiment to experiment. In this case trichloroacetic acid-insoluble counts were determined by the method described for polymerase assay. ^{32}P solubilized by the enzyme was also determined by measuring Cerenkov radiation in the filtrate.

Measurement of degradation during synthesis. Conditions were the same as for the polymerase assay except that the reaction was stopped with 25 μl of 0.1 M EDTA per 0.2 ml of incubation mixture. To measure template-dependent conversion of [^{3}H]dTTP to [^{3}H]dTMP, 10 μl of this reaction mixture was applied to a 3- by 25-cm PEI plate, and ascending chromatography was run with 1.0 M LiCl as the solvent (19). After the solvent front had moved about 20 cm, the plates were taken out and dried, and portions containing the dTMP and dTTP spots (as seen under UV) were scraped into 2 ml of 1 M NH_4OH. Cold dTMP and dTTP were used as markers. After 30 min the supernatant was placed in another tube and dried under vacuum in an Evapomix (Buchler Instruments). This was then suspended in 1.5 ml of water and counted in a dioxane-based scintillator {2.75 kg of naphthalene, 12.6 g of PPO [2,5-diphenyloxazole], and 0.9 g of di-Me-POPOP [1,4-bis-(5-phenyloxazolyl)] dissolved in 18 kg of dioxane}. To estimate percent recovery, [^{32}P]-dTMP was used as an internal marker; recovery was found to be 60% consistently. DNA synthesis and degradation of the template in such experiments were determined as described above.

Nicking and denaturation of ^{32}P-labeled T7 DNA. Usually in the studies described here nicked or nicked and then denatured ^{32}P-labeled T7 DNA was used. For nicking native ^{32}P-labeled T7 DNA, conditions were the same as those for exonuclease assay except that approximately 130 nmol of ^{32}P-labeled T7 DNA (nucleotide P basis) was used per 0.3 ml of incubation mixture. Pancreatic DNase was diluted in 0.05 M Tris (pH 7.6) containing 5 mM $CaCl_2$ to a concentration of 0.2 μg/ml immediately before use, and 10 μl of this was used per 0.3 ml of incubation mixture. After 15 min at 37°C, the enzyme was inactivated by keeping the incubation mixture at 75°C for 5 min and then cooling (10). Denaturation was carried out by keeping DNA solutions in a boiling-water bath for 5 min and then cooling rapidly.

Micrococcal nuclease and alkaline phosphatase treatment. In these experiments 0.3 ml of incubation mixture contained 100 nmol of ^{32}P-labeled T7 DNA, 33 mM $(NH_4)HCO_3$ (pH 8.2), 2 mM $CaCl_2$, and 0.018 U of micrococcal nuclease. This was incubated for 20 min at 37°C and then kept in a boiling-water bath for 5 min and rapidly cooled. This treatment inactivated the enzyme and also denatured the DNA (26).

Part of the DNA obtained from the above procedure was treated with alkaline phosphatase at 65°C for 5 min under conditions used for exonuclease assay. For about 35.5 nmol of ^{32}P-labeled T7 DNA, 1.5 U of alkaline phosphatase was used (24).

Polyacrylamide gel electrophoresis. Native and gel electrophoresis were done under conditions described by Fujimura and Roop (10).

To detect enzyme activities in the native gel, runs were carried out at 4°C. After the run, the gel was sliced longitudinally. One half was stained as described by Weber and Osborn (23). The other half was sliced transversely into many slices, which were placed in individual tubes; to each of these was added 0.1 ml of the enzyme dilution buffer described above. The tubes were kept overnight at 4°C, and the next day assays were done with the eluate for polymerase and exonuclease.

Product identification. A method described by Frenkel and Richardson (7) was used to identify the exonuclease product. The reaction was stopped, after incubation as described above, by adding 0.3 ml of cold 10% trichloroacetic acid containing 0.01 M $Na_4P_2O_7$ and 0.2 ml of 1 mg of native calf thymus DNA per ml to 0.2 ml of the incubation mixture. The precipitate was centrifuged down, and the supernatant was collected. This supernatant was extracted three times with ether to remove trichloroacetic acid and then applied on a Whatman no. 3 filter paper. Descending chromatography was run using the 1-propanol-NH_4OH-water solvent system of Hanes and Isherwood (12). After about a 50-h run the paper was dried, cut into 2-cm strips, and counted as described above.

The ether-extracted supernatant was treated with 5'-nucleotidase as follows. The pH of the solution was brought to about 8.0 by the addition of NaOH. The solution was then made 0.1 M glycine-NaOH (pH 8.0) and 0.01 M $MgCl_2$. This mixture was treated with 0.6 U of 5'-nucleotidase (*Crotalus adamenteus* venom, Sigma) for 1 h at 37°C (13). The product thus obtained was analyzed by ascending paper chromatography using DE81 (Whatman) paper and 2 M CH_3COOH + 0.05 M citric acid made to pH 3.0 by the NaOH solvent system described by Forgách et al. (6). This paper chromatography system separates deoxynucleosides and dNMP's. ^{3}H-labeled DNA was used for 5'-nucleotidase treatment because inorganic phosphate and dNMP's comigrated under the conditions used. 3'-dNMP's were

prepared according to a method described by Fujimura (9) and treated with 5'-nucleotidase as described for 5'-dNMP's. After about an 8-h run the paper was dried, cut into 2-cm strips, and counted as described above.

RESULTS

Purity of the enzyme preparation and dependence of the rate of degradation of ^{32}P-labeled T7 DNA on enzyme concentration. The T5 DNA polymerase purified by the method of Fujimura and Roop (10) gave single bands on both native and sodium dodecyl sulfate-gel electrophoresis, indicating that the preparations were homogeneous. When nicked ^{32}P-labeled T7 DNA was incubated with various amounts of enzyme, the rate of release of [^{32}P]-dNMP was found to be proportional to the amount of enzyme (data not shown).

Association of polymerase and exonuclease activities with the same proteins. To see whether the exonuclease activity present in our preparations of polymerase is associated with the same protein, polyacrylamide gel electrophoresis was run at 4°C, and slices were assayed as described in Materials and Methods. We found that both the activities coincided with the protein peak (Fig. 1), which indicated that the exonuclease and polymerase activities were associated with the same protein. Another piece of evidence that supports our findings is that purified DNA polymerase from T5 *ts*53, a T5 mutant in which DNA synthesis is temperature sensitive (L. Yu, Ph.D. thesis, Emory Univ., Atlanta, Ga., 1968), has been reported to show temperature sensitivity when denatured T7 DNA is used as template. This mutant enzyme also shows a much higher exonuclease activity at higher nonpermissive temperature than does the wild-type enzyme. A revertant of this mutant had temperature-stable polymerase and normal exonuclease activity (R. K. Fujimura, Fed. Proc. 33:1492, 1974). Thus, both the activities are apparently affected by a mutation in the structural gene of the T5 polymerase (4).

Substrate specificity. The enzyme degraded both single- and double-stranded DNA. The apparent rate for denatured T7 DNA was about five times faster than that for native T7 DNA (Fig. 2). The faster rate with single-stranded DNA suggests that the enzyme might be involved in clipping single-stranded tails in DNA which might arise in vivo.

pH optimum. The exonuclease activity was found to have a pH optimum at 8.6, which is identical to the pH optimum for polymerase activity (data not shown). On the lower pH side the rate showed a typical sigmoidal rise, which indicated that a group of pK_a 6.8 might be involved in catalysis. On the other side the rate fell sharply after pH 9.4, presumably because of inactivation of the enzyme.

Dependence of rate of hydrolysis on Mg^{2+}. The enzyme showed an essential requirement of Mg^{2+} for its exonuclease activity, as in the case of polymerase activity. Therefore, in our experiments Mg^{2+} was usually kept at 6.7 mM, as mentioned earlier. The rate of exonuclease action remained constant in a range from 1.6 to 16 mM (data not shown). Both the polymerase and exonuclease activities were totally inhibited by EDTA.

Effect of DTT on exonuclease. The presence of DTT in the reaction mixture and throughout the purification steps was found to be desirable. Incubations run without DTT showed a gradual inactivation of the enzyme, as evidenced by a decrease in the rate of hydrolysis. *N*-ethylmal-

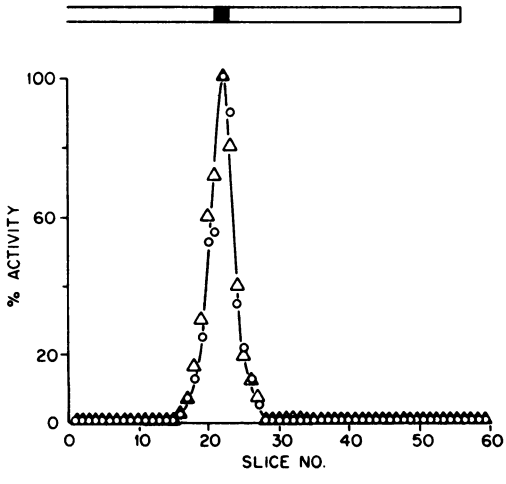

FIG. 1. *Coelectrophoresis of DNA polymerase and exonuclease activities with the protein band. Symbols: ○, polymerase activity; △, exonuclease activity.*

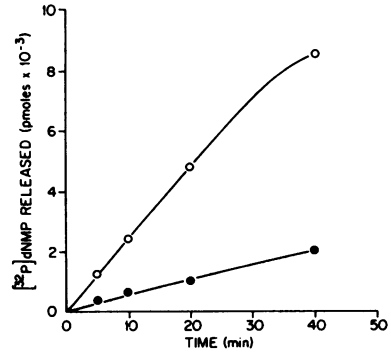

FIG. 2. *Relative rates of degradation of native (●) and denatured (○) T7 DNA. Saturating amounts of substrates were used.*

eimide almost completely inhibited the enzyme. However, when *N*-ethylmaleimide was added in the presence of DTT, there was no effect on the activity (Fig. 3). Polymerase activity was similarly inhibited by *N*-ethylmaleimide (data not presented). These observations indicated the presence of free —SH groups in the enzyme, essential for maximal activity.

Kinetic constants of the exonuclease. As mentioned earlier, the rate of degradation of denatured DNA was much faster than the rate for native DNA, which indicates that the apparent V_{max} values differed by a factor of 5. Examination of the dependence of exonuclease action on DNA concentration showed that the enzyme apparently had a higher affinity for denatured DNA. The apparent K_m for nicked T7 DNA was 21.9 μM, and for nicked and denatured T7 DNA it was 15.0 μM on a nucleotide P basis (Fig. 4A and B). The apparent K_m values were obtained from the nonweighted, linear, least-squares fit of the data. Apparent V_{max} values corresponded to turnover numbers of 10 and 46 mol of dNMP hydrolyzed/min per mol of enzyme for nicked T7 DNA and nicked and denatured T7 DNA, respectively.

Mode of action of exonuclease. The evidence presented below indicates that the enzyme was a 3′→5′ exonuclease. The enzyme seems to produce 5′-dNMP attacking from a 3′-OH end.

Mode of action. (i) Enhancement of the rate of hydrolysis by pancreatic DNase treatment. Native T7 DNA, as shown earlier, was degraded rather slowly by the exonuclease. However, when ^{32}P-labeled T7 DNA was treated with pancreatic DNase, prior to its use as substrate, the rate increased considerably. Pancreatic DNase is known to produce single-stranded nicks in native double-stranded DNA, producing 3′-OH and 5′-PO_4 terminals. Thus, the increased rate of degradation might be explained by invoking either 3′-OH or 5′-PO_4 as the new exonuclease-sensitive sites. With increasing amounts of pancreatic DNase used for nicking, there was a greater increase in the rate of hydrolysis, as expected, when excess enzyme was used (Fig. 5).

(ii) Effect of micrococcal nuclease treatment on the rate of hydrolysis. Micrococcal nuclease is known to produce single-strand nicks in native double-stranded DNA, generating 3′-PO_4 and 5′-OH terminals. When native T7 DNA was treated with micrococcal nuclease and denatured, the rate of degradation was not very different from that of intact denatured T7 DNA. However, when the micrococcal-nuclease-treated and then denatured T7 DNA was

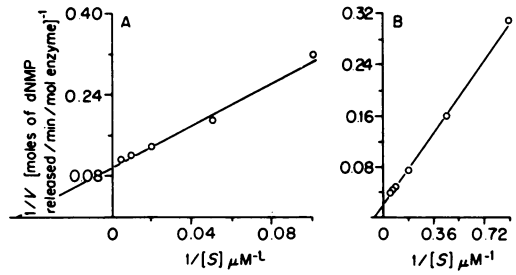

FIG. 4. *Dependence of rate of exonuclease action on substrate concentration; Lineweaver-Burk plots. (A) Nicked ^{32}P-labeled T7 DNA. (B) Nicked and then denatured ^{32}P-labeled T7 DNA. The lines are obtained by nonweighted, linear, least-squares fit of the data.*

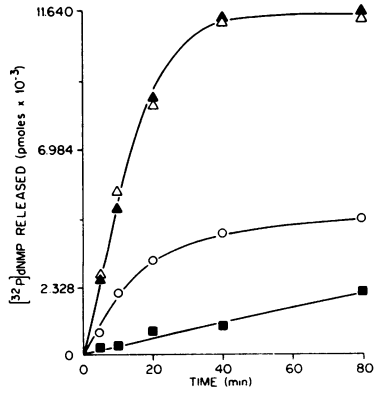

FIG. 3. *Effect of DTT and N-ethylmaleimide (NEM) on exonuclease. Symbols: ○, control; ■, NEM (5 mM); △, DTT (17.8 mM); and ▲, DTT (17.8 mM) + NEM (5 mM). Incubation mixture, 0.1 ml.*

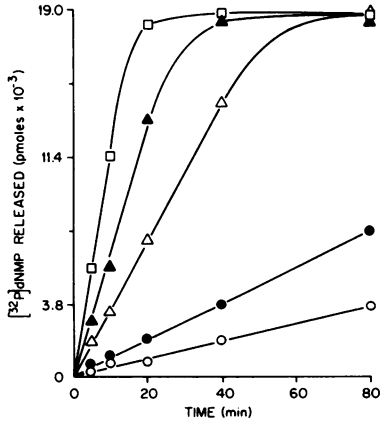

FIG. 5. *Degradation of ^{32}P-labeled T7 DNA with various numbers of nicks. Pancreatic DNase was used as follows: ○, control; ●, 1 ng; △, 2 ng; ▲, 4 ng; and □, 20 ng. Details are given in Materials and Methods. Incubation mixture, 0.2 ml.*

treated with alkaline phosphatase prior to its use as substrate, the rate of degradation increased considerably (Fig. 6). Alkaline phosphatase treatment of intact denatured T7 DNA, however, did not produce any change. These observations indicate that the enzyme is a 3′→5′ exonuclease and shows specificity toward the 3′-OH end.

(iii) Degradation of newly synthesized DNA before old template. It is well known that synthesis of new DNA takes place by the addition of mononucleotides at the 3′-OH end of the primer template. Based on this fact, we designed an experiment as follows. Nicked ^{32}P-labeled T7 DNA was used as primer template for the synthesis of new [^{3}H]dTMP-labeled DNA by T5^{+} polymerase. This product DNA, containing both ^{3}H and ^{32}P labels, was used as substrate for exonuclease assay. If the enzyme is 3′→5′ exonuclease, then there might be a detectable lag in the appearance of trichloroacetic acid-soluble ^{32}P as compared with the control, and this lag should be correlated with the disappearance of trichloroacetic acid-insoluble [^{3}H]dTMP. This is exactly what we found. The DNA containing both ^{3}H and ^{32}P labels showed a lag in the appearance of trichloroacetic acid-soluble [^{32}P]dNMP. However, [^{3}H]dNMP release did not show any lag. After some time, the rate of release of [^{32}P]-dNMP increased and became equal to that of the control, which was correlated with almost total disappearance of ^{3}H label from the trichloroacetic acid-insoluble fraction (Fig. 7). However, on comparing the amounts degraded, the newly synthesized strand was degraded at a much slower rate than the ^{32}P-labeled and nicked T7 DNA. We believe that this difference is due to the presence of the strand, which was displaced during synthesis and might provide a steric hinderance to exonuclease binding at the 3′-OH end. It has been shown by Fujimura and Roop (10) that T5^{+} polymerase causes strand displacement during synthesis when nicked T7 DNA is used as template.

Degradation of the template during synthesis. Goulian et al. (11) reported that degradation of the primer-template DNA by T4 polymerase was inhibited in the presence of required dNTP's, i.e., under synthetic conditions. It was later shown by various authors that, as expected, degradation was inhibited due to the new synthesis, which leaves the primer-template 3′-OH end behind. We investigated this phenomenon with nicked ^{32}P-labeled T7 DNA and found that under synthetic conditions the degradation of the template was undetectable. Controls run without 4dNTP's, however, showed considerable degradation under similar conditions (Fig. 8A). On the other hand, when nicked and denatured ^{32}P-labeled T7 DNA was used as template, there was an initial phase of

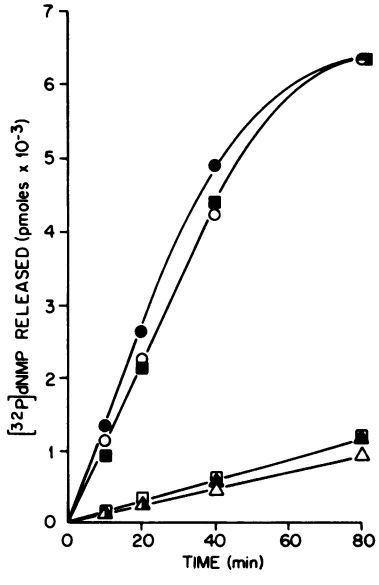

FIG. 6. *Degradation of ^{32}P-labeled T7 DNA after pancreatic and micrococcal DNase treatment. Symbols: △, denatured T7; ▲, treated with micrococcal nuclease and then denatured; ○, treated with pancreatic DNase and denatured; ●, treated with alkaline phosphatase after treatment with micrococcal nuclease and denaturation; □, denatured T7 treated with alkaline phosphatase; ■, treated with alkaline phosphatase after treatment with pancreatic DNase and denaturation. Incubation mixture, 0.2 ml.*

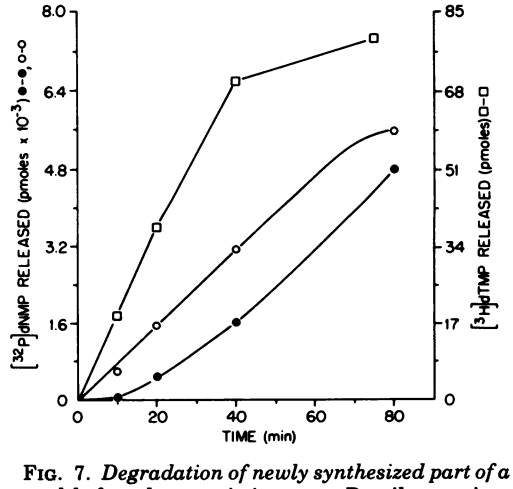

FIG. 7. *Degradation of newly synthesized part of a strand before the preexisting part. Details are given in Results. Symbols: ○, control; ○, ●, [^{32}P]dNMP; □, [^{3}H]dTMP.*

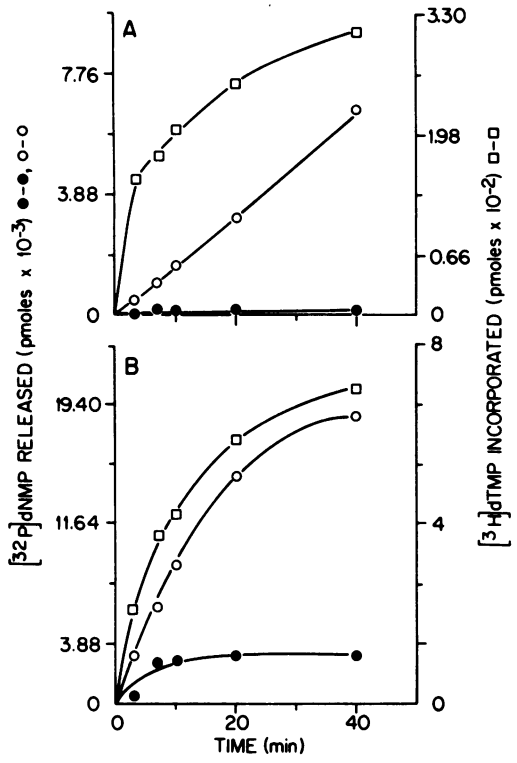

FIG. 8. *Degradation of template during synthesis. (A) ^{32}P-labeled nicked T7 DNA; (B) ^{32}P-labeled nicked and then denatured T7 DNA. Symbols: ○, degradation in absence of 4dNTP; ●, degradation in presence of 4dNTP; □, incorporation.*

degradation of the template which stopped as the reaction proceeded; the polymerization, however, went on smoothly (Fig. 8B). The initial phase of primer-template degradation in this case can be assigned to the removal of unhybridized 3'-OH tails of hairpin-type structures (5), until the double-stranded region is reached, where polymerization begins.

Degradation of newly synthesized strand during synthesis. Under synthetic conditions we found that newly incorporated nucleotides were hydrolyzed. Degradation was followed by the method of Nossal and Hershfield (17), which consisted of template-dependent conversion of dNTP to dNMP, presumably due to incorporation and subsequent removal. These results are presented in Fig. 9. Controls were run without template and without enzyme. Newly incorporated nucleotides were found to be hydrolyzed in the case of nicked T7 DNA template also (data not presented).

5'→3' Exonuclease activity. 5'→3' Exonuclease activity was tested by using ^{32}P-labeled 5'-T7 DNA (native) and ^{32}P-labeled 5'- and ^{3}H-labeled 3'-T7 DNA (denatured) as substrates. Conditions of incubation were as described for exonuclease. ^{32}P-labeled 5'-T7 DNA (native) was made trichloroacetic acid soluble after a considerable lag (Fig. 10A). In the case of denatured T7 DNA, ^{3}H label from the 3'-end became trichloroacetic acid soluble first, followed by ^{32}P label from the 5'-end (Fig. 10B). These results indicated that under our assay conditions 5'→3' exonuclease activity was undetectable.

Endonuclease activity. The enzyme preparation was tested for the presence of endonucle-

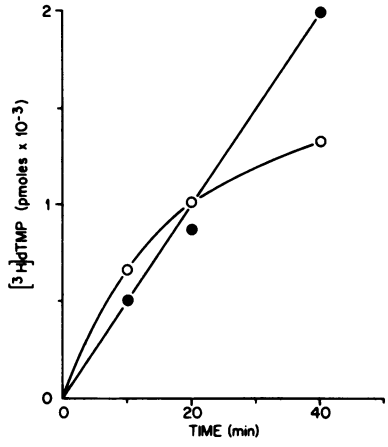

FIG. 9. *Hydrolysis of newly incorporated nucleotides during synthesis. Details are given in Materials and Methods. Symbols: ○, incorporation; ●, hydrolysis. Nicked and then denatured T7 DNA was used as template.*

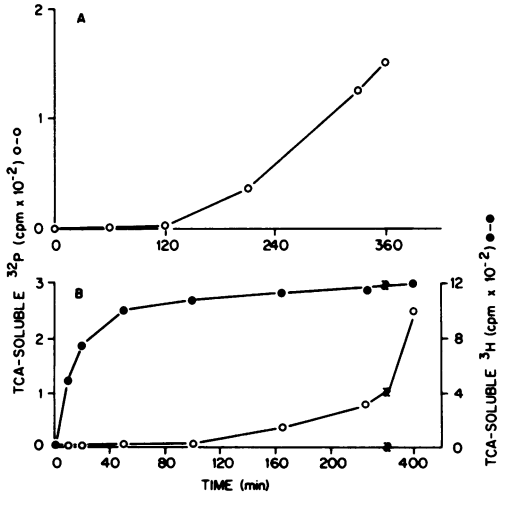

FIG. 10. *Degradation of ^{32}P-labeled 5'-T7 DNA by T5 polymerase. (A) Native T7 DNA; (B) denatured T7 DNA (also labeled with ^{3}H at the 3'-end). Specific activity of ^{32}P-5' label was 100 cpm/μg of DNA in both cases.*

ase activity with ^{3}H-labeled DNA from bacteriophage PM2. ^{3}H-labeled PM2 DNA was treated with the enzyme under optimal conditions for exonuclease, and the reaction mixture was put on an alkaline sucrose gradient. The pattern was found to be essentially similar to the control without enzyme (data not shown). Thus, endonuclease activity was undetectable.

Identification of exonuclease products. With both denatured (Fig. 11A) and nicked (Fig. 11B) T7 DNA, 95% of the product was found under the mononucleotide peak. About 5% of the total product was dinucleotide (15). Thus, it appears that the exonuclease primarily produces dNMP's and a small amount of dinucleotides. Since no endonuclease activity was detected with ^{3}H-labeled DNA from PM2 phage, it appears likely that the dinucleotides are also produced by the exonucleolytic activity. We are investigating this point in detail.

Upon 5'-nucleotidase treatment of the exonuclease product, essentially all the dNMP's were converted to deoxynucleosides (Fig. 12). The 5'-nucleotidase preparation has no detectable 3'-nucleotidase activity (Fig. 13), since the 3'-dNMP's were not converted to deoxynucleosides. These findings indicate that the major product of exonuclease action is 5'-dNMP.

DISCUSSION

Various DNA polymerases have been shown to have 3'→5' exonuclease activity associated with them. It has been suggested by several authors that this activity might be crucial for faithful replication, as it might be involved in removing misincorporated bases. As has been shown in this paper, T5 polymerase has a 3'→5'

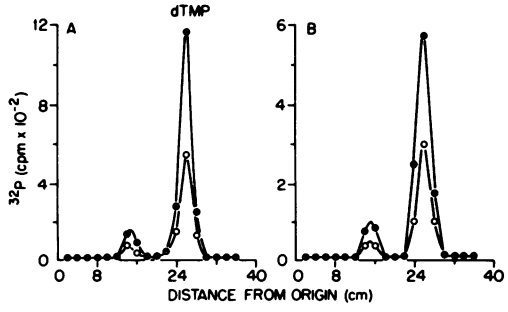

FIG. 11. *Product identification by paper chromatography. (A) Denatured T7 DNA as substrate. Symbols: ○, 40 min; ●, 80 min (times of incubation). A total of 18 and 36%, respectively, of the substrate was made trichloroacetic acid soluble. (B) Nicked T7 DNA as substrate. Symbols: ○, 40 min; ●, 80 min (times of incubation). A total of 12 and 25%, respectively, of the substrate was made trichloroacetic acid soluble.*

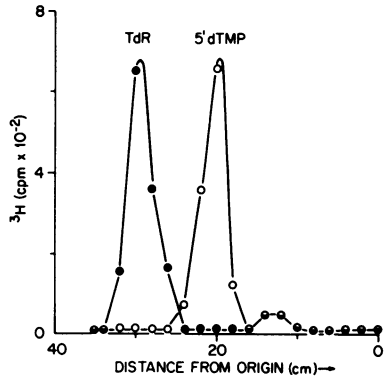

FIG. 12. *DE81 paper chromatography of exonuclease product. Symbols: ○, control; ●, treated with 5'-nucleotidase.*

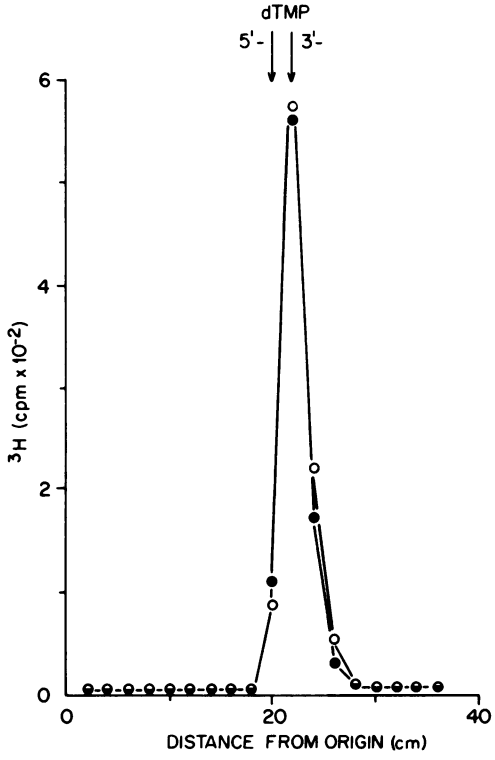

FIG. 13. *DE81 paper chromatography of 3'-dNMP's. Symbols: ○, control; ●, treated with 5'-nucleotidase.*

exonuclease activity associated with it. It is reasonable to consider the possibility that the residence of these two activities on the same protein molecule makes the system much more efficient and error free. However, to date no evidence has been presented to indicate that this activity is essential for replication.

The observation that single-stranded DNA is degraded much faster than duplex DNA indi-

cates that this enzyme might be involved in trimming 3'-OH tails that might occur as a result of various recombinational processes. A limited degradation of the denatured DNA template during synthesis also suggests a similar possibility. The requirements for polymerase and exonuclease activities were remarkably similar, which indicates that they might have at least some overlap between their catalytic repertoires. A very interesting possibility is that they have a common active site.

Under synthetic conditions, though the template was found to be protected, the newly synthesized strand was found to be turned over during synthesis. This was shown by template-dependent conversion of [^{3}H]dTTP to [^{3}H]-dTMP. Why the newly synthesized DNA should be degraded so extensively in spite of the availability of 4dNTP's still remains a question. It is possible that there are factors in vivo that would reduce this turnover considerably.

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