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Mechanism of 3'→5' exonuclease associated with phage T5-induced DNA polymerase: processiveness and template specificity

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ABSTRACT

T5-induced DNA polymerase has an associated 3'→5' exonuclease activity. Both single-stranded and duplex DNA are hydrolyzed by this enzyme in a quasi-processive manner. This is indicated by the results of polymer-challenge experiments utilizing product analysis techniques. Due to the quasi-processive mode of hydrolysis, the kinetics of label release from the 3'-terminally labeled oligonucleotide substrates, annealed to complementary homopolymers, show an initial high rate of hydrolysis. In the case of both single-stranded and duplex DNA substrates, hydrolysis seems to continue, at best, up to the point where the enzyme is five or six nucleotides away from the 5'-end.

The enzyme carries out mismatch repair, as evidenced by experiments with primer molecules containing improper base residues at the 3'-OH terminus. Control experiments with complementary base residues at the 3'-end indicate that extensive removal of terminal residue takes place in the presence of dNTP's only when such residues are "improper" in the Watson-Crick sense.

In our previous publications we have noted that bacteriophage T5-induced DNA polymerase replicates DNA in a processive fashion (1,2). This imparts biphasicity to the kinetics of DNA synthesis in vitro when short primer-templates are used as substrate. The enzyme continues to replicate a given primer-template until it is very close to the 5'-end of the template (1; unpublished observations). T5 polymerase possesses a 3'→5' exonuclease activity which utilizes single-stranded, duplex, denatured, and nicked DNA as substrates (3a,b); this enzyme is devoid of any 5'→3' exonuclease activity. Here we present evidence demonstrating that the mode of action of exonuclease activity is "quasi-processive," as defined in (2). Both single-stranded and duplex DNA substrates are hydrolyzed in this manner. After hydrolysis of a given substrate molecule is initiated at the 3'-end, the enzyme continues to hydrolyze the molecule until it is five to six nucleotides away from the 5'-end. The oligonucleotides

released from the 5'-end of the substrate are eventually degraded to the limit dinucleotide.

In the prokaryotic system there are many data suggesting that mutations in a structural gene for polymerases affect the spontaneous mutation rate of an organism, and that the polymerase-associated 3'→5' exonuclease activity plays a role in the reduction of the mutation rate (4). Therefore we have carried out experiments similar to those of Brutlag and Kornberg (5) to see whether the 3'→5' exonuclease preferentially removes mismatched bases. These experiments indicated that the 3'→5' exonuclease activity does remove mismatched residues at 3'-OH termini before it initiates primer elongation.

MATERIALS AND METHODS

Enzymes. T5-induced DNA polymerase was purified according to the procedure of Fujimura and Roop (6). Native- and sodium dodecyl sulfate-gel electrophoretic analysis showed that the enzyme preparations were homogeneous. Both polymerase and exonuclease activities were assayed with denatured T7 DNA and nicked T7 DNA, and all the activities coincided with the protein peak (3a,b, 6, and unpublished observations). Endonuclease and 5'→3' exonuclease activities were assayed with ^{3}H-labeled PM2 DNA and [5'-^{32}P] poly(dA), respectively, and there was no indication for the presence of these contaminating activities. T4-induced polynucleotide kinase and calf thymus terminal transferase were obtained from P-L Biochemicals. Micrococcal nuclease and spleen phosphodiesterase (Worthington) were essentially free of 3'-nucleotidase activity (7). E. coli DNA polymerase I was purified according to the method of Jovin et al. and had a specific activity of 6000 U/mg protein with activated calf thymus DNA (8).

Substrates. Calf thymus DNA was from Calbiochem; poly(dA), poly(dC), and sheared calf thymus DNA had a number-average chain length of ~300, ~180, and ~600 residues, respectively, as determined by the polynucleotide kinase method described by Richardson (9). Poly(dA), poly(dC), oligo(dT)$_{12-18}$, oligo(dT)$_{10}$, and four dNTP's were obtained from P-L Biochemicals. [Methyl-^{3}H] dTTP and [5-^{3}H] dCTP were from Schwarz/Mann. [γ-^{32}P] ATP was supplied by New England Nuclear. [^{3}H] poly(dA) (Miles) had a number-average chain length of ~150 as determined by 3'-end analysis by use of micrococcal nuclease and spleen phosphodiesterase, described

by us earlier (1). [^{3}H] poly(dT)$_{\overline{220}}$, [^{3}H] poly(dC)$_{\overline{200}}$, and [^{3}H] poly(dA)$_{\overline{300}}$ were prepared by Bollum's method with oligo(dT)$_{\overline{12-18}}$, oligo(dC)$_{\overline{12-18}}$, and oligo(dA)$_{\overline{12-18}}$, respectively, as primer (10).

Labeled nucleotides were added to oligonucleotides according to the method of Bollum (10). The oligonucleotides were separated from labeled precursors on a Sephadex G-50 column (80 X 1.4 cm) and washed with 0.01 M Tris-HCl (pH 7.6) containing 0.1 mM EDTA. The early-emerging radioactivity peak was collected, dried by flash evaporation, and dissolved in a desired volume of H_2O. For the calculation of the average number of labeled residues per primer, the primer length was assumed to be 15. Average lengths of labeled segments were also confirmed by micrococcal nuclease and spleen phosphodiesterase digestion of the oligonucleotides (1).

For the preparation of ^{3}H-labeled calf thymus DNA and poly(dA)$_{\overline{300}}$:[^{3}H] poly-(dT)$_{\overline{150}}$, sheared and denatured calf thymus DNA and poly(dA)$_{\overline{300}}$:oligo(dT)$_{\overline{12-18}}$ were used as primer templates, respectively. In both cases, the labeled nucleotide was [methyl-^{3}H] dTTP. E. coli DNA polymerase I was used for the preparation of these substrates. Separation of labeled polymers from precursors was carried out by gel filtration as indicated above. The length of the labeled segment of these substrates was determined by converting these polymers to 3'-deoxynucleotides and deoxynucleosides as noted above (1). In the case of calf thymus DNA, it was assumed that 25% of the labeled molecules had a thymine residue at the 3'-OH end.

Conditions for exonuclease assay. Each 0.3 ml of the reaction mixture contained the following: Tris-HCl (67 mM, pH 8.6), 6.7 mM $MgCl_2$, 17 mM dithiothreitol, and indicated amounts of DNA substrate. The reaction was usually initiated by the addition of enzyme unless otherwise indicated. At desired time intervals, aliquots were taken out and spotted on DE81 paper squares. These were dried and placed in a scintillation vial, then eluted with 1.0 ml of 0.3 M NH_4-formate by gentle shaking. After removal of the eluate, they were again eluted with 0.5 ml of the same solution. These two eluates were collected, mixed with 15 ml of ACS (Aqueous Counting Scintillant, Amersham/Searle), and counted in a Packard Tri-Carb liquid scintillation counter. Zero-minute blanks obtained by this assay were 0.2–0.5% of the input counts. For 5'-^{32}P-labeled substrates without template, the blank values were in the range of 3–5%.

Trichloroacetic acid precipitation and subsequent processing of reaction mixtures, wherever indicated, were carried out essentially as described earlier (3a).

RESULTS

Processiveness of 3'→5' exonucleolytic activity of T5-induced DNA polymerase.

The time course of hydrolysis of oligonucleotides was studied under conditions of an excess of substrate over the T5 enzyme. The substrate was poly(dA)$_{\overline{300}}$:oligo(dT)$_{\overline{12-18}}$·[^{3}H](dT)$_{\overline{3.0}}$. The experiments were carried out at 25°C (Fig. 1A) and 15°C (Fig. 1B), and the results suggest a biphasic curve with a fast initial rate followed by a slower final rate.

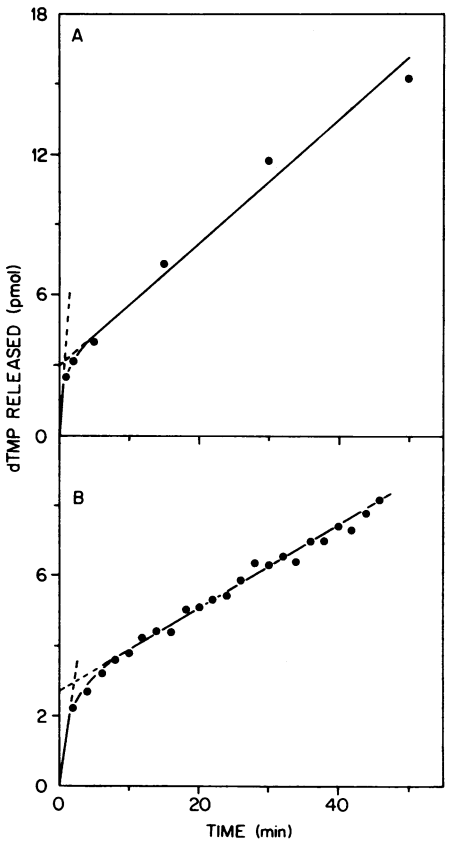

Fig. 1. Hydrolysis of poly(dA)$_{\overline{300}}$:oligo(dT)$_{\overline{12-18}}$·[^{3}H](dT)$_{\overline{3.0}}$ by T5 DNA polymerase. (^{3}H specific activity was 400 cpm/pmol.) Final poly(dA)$_{\overline{300}}$ and oligo(dT)$_{\overline{12-18}}$. [^{3}H](dT)$_{\overline{3.0}}$ concentrations are 200 nM (5'-end basis). Other conditions are detailed in the text. 1 pmol of T5 enzyme was used for each time point. Temperatures of incubation were (A) 25°C and (B) 15°C. 100-µl aliquots were taken out for each time point. Blank was about 120 cpm, which is ~0.5% of the input counts.

Similar biphasic curves were obtained previously for the polymerization reactions, and the initial fast rate was shown to be due to processiveness of the reaction (1). Thus the hydrolytic reaction was interpreted similarly. Therefore the number of labeled residues hydrolyzed by the end point of the initial phase is due to a processive process by the enzyme molecules. Such end points are at the intersection of tangency drawn contiguous with the initial rate and the final rate. With a given primer, the location of this point on the ordinate is directly proportional to the amount of enzymes, as shown to be true in Fig. 2.

To obtain some direct evidence for the processive hydrolysis by the enzyme we carried out polymer-challenge experiments using three kinds of substrates — poly(dA): oligo(dT), poly(dA):poly(dT), and denatured calf thymus DNA. Enzyme was preincubated with a labeled DNA substrate under the same conditions as for exonuclease assay, except that Mg^{2+} was not added. Under these conditions the enzyme forms a complex with a primer end of DNA substrate (7). When Mg^{2+} is added to such reaction mixtures, hydrolysis of DNA is initiated with biphasic time course. When a competing polymer is included with Mg^{2+} it causes no perceptible change in the initial rate of hydrolysis as shown in Fig. 3A-C for all three substrates. Later, however, the apparent rate of hydrolysis is substantially altered for all three substrates. These results show that the enzyme continues to hydrolyze a given substrate molecule for some time before it dissociates. These experiments, however, do not clearly show the effect of structure

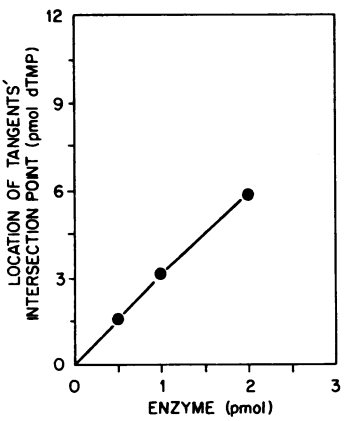

Fig. 2. Dependence of the location of the point of intersection of the tangents on the amount of enzyme used. Conditions are as in Fig. 1A.

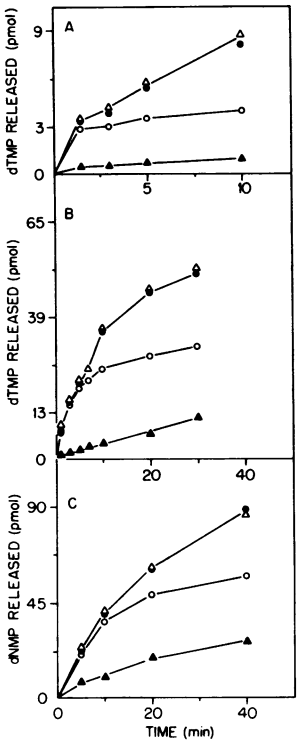

Fig. 3. Polymer challenge experiments: These experiments were carried out with (A) poly(dA):oligo(dT), (B) poly(dA):poly(dT), and (C) denatured calf thymus DNA. Challenger polymers were the same as test substrates except unlabeled. The enzyme was preincubated with various DNA for 1 min in the presence of all the ingredients needed for exonuclease action except Mg^{++}. As an additional safeguard, 0.5 mM EDTA was also included. The reaction was initiated by addition of Mg^{2+} to final net concentration of 6.6 mM at 0 min. (A) ●, Preincubation was started at -1 min with poly(dA)$\overline{300}$:(dT)$\overline{12-18}$·[^{3}H](dT)$\overline{3.0}$ (1:1 molar basis) (primer concentration, 300 nM). ○, Preincubation as for ●; reaction was started by the addition of poly(dA)$\overline{300}$:(dT)$\overline{12-18}$ (3.0 μM) with Mg^{2+}. △, As for ● except that poly(dA)$\overline{300}$:(dT)$\overline{12-18}$·[^{3}H](dT)$\overline{3.0}$ (3.0 μM) was added with Mg^{2+}. ▲, Poly(dA)$\overline{300}$:(dT)$\overline{12-18}$ (3.0 μM) was added at -1 min; otherwise as for ●. In this set of experiments 1 pmol enzyme was used for each time point. (B) ●, Poly(dA)$\overline{300}$:[^{3}H] poly(dT)$\overline{150}$ concentration was 300 nM (5'-end basis) during the preincubation. ○, As for ● except that reaction was started by the addition of Mg^{2+} + poly(dA)$\overline{300}$:poly(dT)$\overline{220}$ (3.0 μM). △, At 0 min, Mg^{2+} + poly(dA)$\overline{300}$:[^{3}H] poly(dT)$\overline{150}$ (3.0 μM) was added. ▲, Cold poly(dA):poly(dT)$\overline{220}$ (3.0 μM) was added at 0 min; otherwise as for ●. In this set of experiments 0.2 pmol enzyme was used for each time point. (C) Combinations are as in (A) and (B) except that initial labeled substrate, denatured calf thymus DNA, concentrations were 460 nM (5'-end basis), and 4.6 μM unlabeled substrates were added as challengers. 0.6 pmol of enzyme was used for each time point. In all these experiments the temperature was 22°C. 100-μl samples were analyzed. Zero-minute blanks were 0.5, 0.21, and 0.28 respectively.

of a substrate on degree of processiveness.

Mode of hydrolysis of single-stranded DNA. With enzyme, the exonucleolytic turnover number for single-stranded DNA homopolymers is as much as two orders of magnitude higher than the corresponding number for duplex DNA substrates (7). This precluded use of polymer competition experiments for the analysis of processiveness with single-stranded substrates and necessitated the employment of alternate techniques. Also, we have not systematically examined whether enzyme forms the putative "Michaelis complex" with single-stranded DNA in the absence of Mg^{2+}. Experiments similar to those of Nossal and Singer (11), for the analysis of the mechanism of ribonucleases, were performed. Briefly, these experiments consist of product analysis at various stages of reaction with single-stranded DNA substrates. If the enzyme is nonprocessive, virtually all the substrate will be utilized by the enzyme simultaneously, and as a result most of the substrate molecules will be reduced in size. On the other hand, if the enzyme is processive, a given substrate molecule will be degraded substantially before the enzyme switches to the next. When products obtained at various stages of hydrolysis of poly(dA)$_{\overline{300}}$ were examined, the radioactivity distribution profile of the Sephadex G-50 eluate was bimodal. In other words, the radioactivity was in the form of either very small or very large material; intermediate-length oligonucleotides were hardly detectable (Fig. 4). These data suggest that single-stranded substrates are also hydrolyzed processively by the T5 enzyme to some degree. Another piece of evidence which indicated that the enzyme was processive to considerable extent with single-stranded DNA substrates was obtained by comparing the time course of hydrolysis of a mixture of poly(dA) labeled with ^{32}P at the 3'-end and poly(dA) uniformly labeled with ^{3}H (Fig. 5). Their rates of hydrolysis were indistinguishable. If the reaction was nonprocessive, ^{32}P label at the 3'-end should have been hydrolyzed much faster.

Extent of processive hydrolysis. The results presented above indicate that both duplex and single-stranded DNA substrates are hydrolyzed by T5 polymerase in a processive fashion. The extent of processive hydrolysis, however, is not revealed by these experiments. In other words, the question that still remains unanswered is whether the enzyme continues to hydrolyze a given substrate molecule until the very end.

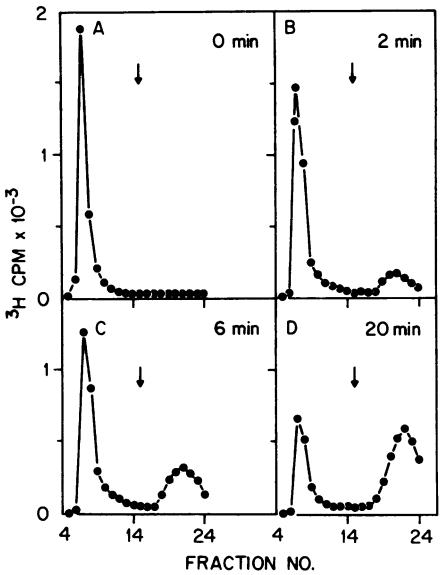

Fig. 4. Analysis of [^{3}H] poly(dA)$\overline{300}$ hydrolysis products by Sephadex G-50 gel filtration. Column dimensions were 27 X 1.4 cm. Column was eluted with 0.01 M Tris-HCl (pH 8.1) + 1 mM EDTA. 1.5-ml fractions were collected every 5 min. For each time point 15 µg of poly(dA)$\overline{300}$ and 1.0 µg of T5 polymerase were used. Incubations were carried out at 25°C. Time of incubation is indicated on each panel. Arrows indicate the position at which poly(dT)$_{10}$ elutes from the column.

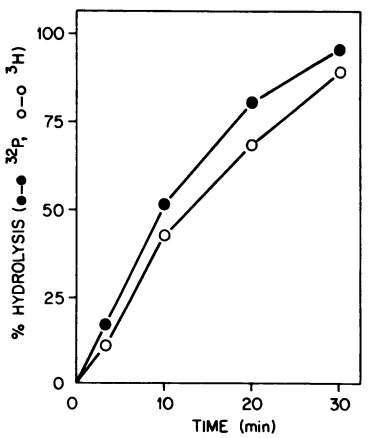

Fig. 5. Hydrolysis of a mixture of poly(dA)$\overline{300}$ · [^{32}P](dA)$\overline{1.0}$ and [^{3}H] poly(dA)$\overline{300}$ by T5 polymerase. Conditions are as in Fig. 4.

To answer this question, we carried out experiments with [5'-^{32}P] oligo(dT)$_{12-18}$: poly(dA)$_{300}$ and [5'-^{32}P] poly(dA)$_{300}$. At various times after the reaction commenced, aliquots were taken out and subjected to DEAE-Sephadex chromatography by use of salt gradients. For product analysis of oligo(dT)$_{12-18}$:poly(dA)$_{300}$, NH_4HCO_3 was used for elution, which did not elute the original substrate by 1 M (Fig. 6A). For [5'-

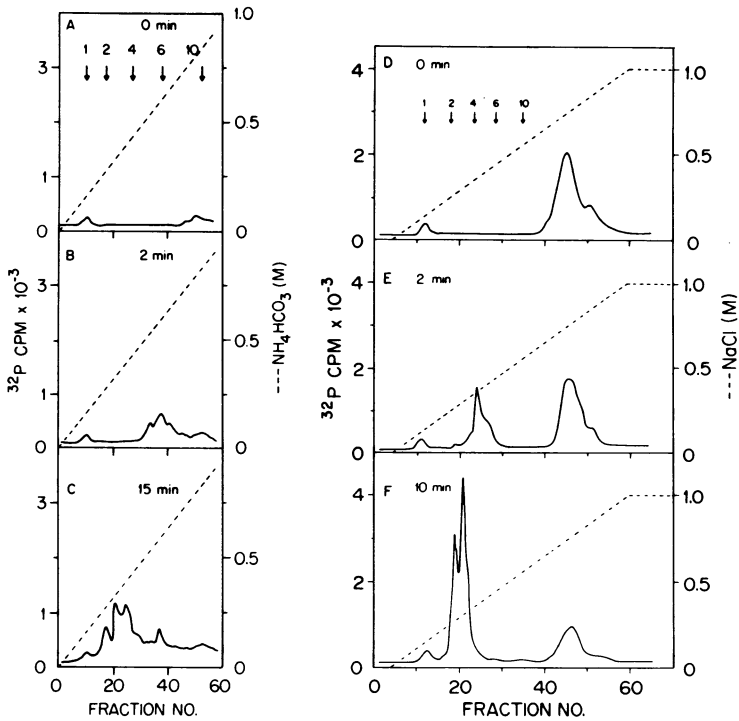

Fig. 6. (A–C) Ion-exchange chromatography of the product obtained with poly(dA)$_{300}$:[5'-^{32}P] oligo(dT)$_{12-18}$ (1:1 molar basis). In a reaction volume of 150 µl, 51.2 pmol of [5'-^{32}P] oligo(dT)$_{12-18}$ and poly(dA)$_{300}$ (5'-end basis) and 6.0 pmol of T5 polymerase were used at 25°C. (D–F) Hydrolysis of [5'-^{32}P] poly(dA)$_{300}$ by T5 polymerase at 22°C. In a reaction volume of 150 µl, 48 pmol poly(dA)$_{300}$ (5'-end basis) and 12 pmol T5 polymerase were used. The following procedure was used in all cases: At indicated times, noted at the right top corner of each panel, 30-µl aliquots were taken out in a tube containing 10 µl of 0.2 M EDTA. After dilution to a volume of 1.0 ml, the products were loaded on a DEAE-Sephadex A-25 column (4.5 X 40 mm) and eluted with a 0.01 M–1.0 M linear NH_4HCO_3 gradient (pH 8.2), dashed line, for (A–C). For (D–F), the products from the column were eluted with 0.01 M–1.0 M NaCl. 50 ml of each solution was used. 1.8-ml fractions were collected. 5'-^{32}P specific activity was ~1600 cpm/pmol.

^{32}P] poly(dA)$_{\overline{300}}$ NaCl was used for salt gradient which eluted the original substrate around 0.7 M (Fig. 6D). NaCl solution is more acidic than NH_4HCO_3, and thus higher polymers were eluted at lower salt concentration. As shown in Fig. 6B, with [5'-^{32}P] oligo(dT)$_{\overline{12-18}}$:poly(dA)$_{\overline{300}}$ the ^{32}P-labeled oligonucleotide released after short periods of incubation is about hexanucleotide. After this, the oligomer probably dissociates from the template and/or the enzyme. In the case of [5'-^{32}P] poly(dA)$_{\overline{300}}$, the length of the residual oligonucleotide is approximately four (Fig. 6E). These experiments indicate that the T5 enzyme is quasi-processive as an exonuclease; it does leave a four- to five-nucleotide-long residue unhydrolyzed during the early phases of the reaction. When incubations were carried out for much longer periods, in both cases the length of the 5'-^{32}P-labeled oligonucleotide was reduced to two (Fig. 6C, 7A and 6F, 7B, respectively).

Effect of DNA structure on the rate of hydrolysis. Although T5 DNA polymerase hydrolyzed both single-stranded and duplex DNA quasi-processively, the relative rate of hydrolysis obtained with various substrates varied by as much as ~ 165-fold. Duplex DNA substrates in general were hydrolyzed much more slowly than the single-stranded substrates. Within the duplex DNA substrate group the rates were very similar (Table I). Among the single-stranded substrates poly(dT)$_{\overline{220}}$ was hydrolyzed much faster (the turnover number at 25°C was ~ 1000/min).

When the temperature of incubation was shifted from 25 to 37°C, the rate of hydrolysis of duplex polymers increased 4- to 6-fold. With poly(dA)$_{\overline{300}}$ there was a dramatic 12-fold increase in the rate of hydrolysis. In the case of poly(dC)$_{\overline{180}}$ and poly(dT)$_{220}$ this increase was rather small (Table I). Studies of optical properties of homopolymers show that poly(dA) has tertiary structures that are highly dependent on temperature and poly(dT) has properties that have small dependence on temperature (11b). Thus the rates of hydrolysis of these homopolymers appear to be affected by tertiary structures.

Proofreading function of 3'→5' exonuclease. For these experiments, oligo(dT) primers containing labeled residues at their 3'-ends were used. As is shown in Fig. 8A, B, these oligonucleotides are hydrolyzed very rapidly by T5 enzyme. The rate of hydrolysis is considerably reduced when they are annealed to poly(dA)$_{\overline{300}}$. We have noted earlier that T5 enzyme shows a marked pereference for the 3'-OH end of the primer molecules, compared with the 3'-OH termini of the template (7). Thus,

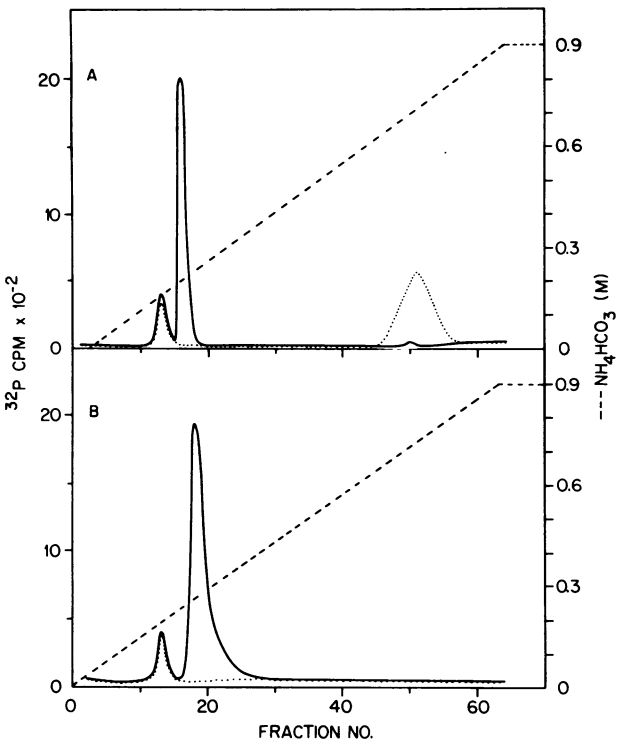

Fig. 7. Limit digests obtained with 5'-terminally labeled substrates. Incubations were carried out as detailed in Fig. 5 and 6, except that with [5'-^{32}P] oligo(dT) $\overline{12-18}$, poly(dA) was not used. Time of incubation in both cases was 80 min at 22°C. Solid line, 0-min sample; dotted line, 80-min sample. (A) [5'-^{32}P] oligo(dT) $\overline{12-18}$; (B) [5'-^{32}P] poly(dA) $\overline{300}$. Analysis was carried out as described in the legend to Fig. 6.

under the conditions used in these experiments, practically all the enzyme molecules, at least initially, are bound at the primer site.

With a "properly" matched (in the Watson-Crick sense) labeled residue at the 3'-end of the primer, the rate of hydrolysis was drastically reduced in the presence of dNTP's (Fig. 8C). Under these conditions, the primer were extended and rendered insoluble in trichloroacetic acid (data not shown).

On the other hand, when the terminally labeled residue was not complementary, the presence of dNTP's did not cause any change in the initial rate of hydrolysis (Fig. 8D). In the presence of dNTP's, the extent of hydrolysis was reduced signifi-

TABLE I Relative rates of hydrolysis for various substrates

Substrate	Rate of hydrolysis (nmol hydrolyzed/ 40 min at 25°C)*	Ratio of rate of hydrolysis 37°/25°
calf thymus DNA†	0.25	—
nicked T5 DNA	0.40	—
nicked and denatured T5 DNA	0.83	—
poly(dA)$_{\overline{300}}$:[^{3}H] poly(dT)	0.58	3.5
poly(dC)$_{\overline{180}}$:[^{3}H] poly(dG)	0.25	5.96
poly(dC)$_{\overline{180}}$	8.7	2.55
poly(dA)$_{\overline{300}}$	6.7	12.31
poly(dT)$_{\overline{220}}$	67.0	1.58

*1.8 pmol of enzyme was used.

†Denatured calf thymus DNA was used as primer-template for E. coli DNA polymerase I for labeling this substrate.

cantly. This is probably due to the processive nature of the enzyme and the limiting amount of enzyme used, compared with the amount of primer, in these experiments. The result also indicates that the rate of hydrolysis is lowered because enzyme molecules are engaged in primer extension. Examination of the trichloroacetic acid-insoluble fraction revealed that none of the primers were extended before the removal of doxycytidine monophosphate residue. Thus, T5 polymerase does not utilize primers containing mismatched residue at the 3'-end.

DISCUSSION

The 3'→5' exonuclease activities of certain bacterial (12, 13), phage-induced (12,3a), and yeast polymerases (14, 15) have been studied by several groups. Biochemical and genetic data that have been collected so far amply reveal that this activity might be involved in the regulation of fidelity of DNA synthesis, as first proposed by

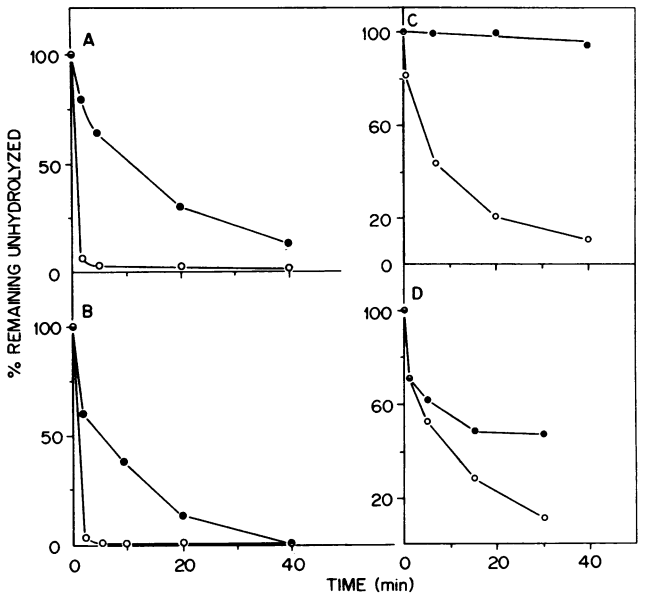

Fig. 8. Hydrolysis of (A) oligo(dT) $\overline{12-18}$·[^{3}H](dT)$\overline{3.0}$ and (B) oligo(dT) $\overline{12-18}$·[^{3}H](dC)$\overline{1.1}$ in the presence (●) and absence (○) of poly(dA)$\overline{300}$. (C) Hydrolysis of matched residue, and (D) hydrolysis of mismatched residue at the 3'-end in the presence (●) and absence (○) of +33 μM dNTP. In a reaction volume of 300 μl, 53.3 pmol of the primer and 132 pmol of template were used. The amount of enzyme used was 8 pmol in all cases. At indicated times after addition of enzyme, 50-μl aliquots were spotted on DE-81 squares and processed as described before. Incubation temperature was 22°C.

Goulian et al. (16). Data presented suggest that 3'→5' exonuclease of T5 polymerase may be also involved in fidelity of replication. Eukaryotic enzymes, in general, do not seem to possess this capability, though there are some claims to the contrary and the matter remains controversial (14–19).

In previous publications from this laboratory, we have shown that T5-induced DNA polymerase is a processive enzyme (1, 20, 2). It continues to replicate a given template until it is one or two nucleotides away from the 5'-end of the template molecule (Das, unpublished observation). When the behavior of this enzyme was compared with that of T4, E. coli, and calf thymus DNA polymerases, it became clear that most other enzymes are "quasi-processive" (2). Another interesting property of this enzyme is that it preferentially binds at the 3'-OH termini of the primer molecules

(7). Nonproductive binding at other locations and binding at the 3'-OH end of the template is minimal in the presence of a sufficient number of primer sites.

Using 3'-terminally labeled oligonucleotides we have shown that the kinetics of label release by T5 enzyme are biphasic. That such a situation will arise, if the enzyme continues to hydrolyze the unlabeled portions of a given primer molecule before switching to the next, is a possibility. Results of competition experiments seem to bear out this prediction. Thus in the case of poly(dA)$_{\overline{300}}$:oligo(dT)$_{\overline{12-18}}$, [^{3}H](dT)$_{\overline{3.0}}$, poly(dA)$_{\overline{300}}$:[^{3}H] poly(dT), and ^{3}H-labeled calf thymus DNA substrates, initial rates of hydrolysis were not altered by the addition of competing polymer. In the subsequent phase of the reaction a precipitous decline in the rate was observed. Also, the rate of hydrolysis of label at the 3'-end was approximately equal to the rate obtained with uniformly labeled substrate. This supports the above interpretation.

Experiments with [5'-^{32}P] poly(dA)$_{\overline{300}}$ indicate that the enzyme continues to degrade a given substrate molecule up to about the fifth nucleotide from the 5'-end. But both [5'-^{32}P] poly(dA)$_{\overline{300}}$ and [5'-^{32}P] oligo(dT)$_{\overline{12-18}}$ yield a terminal dinucleotide as the limit product. Thus it appears that the enzyme continues to hydrolyze a given primer molecule only up to about the sixth nucleotide from the 5'-end. After this, the primer-template complex probably dissociates and hydrolyses to dinucleotide by a slower process. These evidences show that the mode of hydrolysis by this enzyme is quasi-processive.

It has been reported recently that E. coli exonuclease I, which is a single strand-specific enzyme, is processive (21). The 3'→5' exonuclease activities of T4 polymerase (22), E. coli DNA polymerase I (21), and Bacillus subtilis polymerase III (13) have been reported to be nonprocessive. Thus T5 enzyme is the only known polymerase to have a quasi-processive 3'→5' exonuclease activity with single-stranded DNA substrates. For duplex substrates also, T5 enzyme is quasi-processive. The only other enzyme known to us which hydrolyzes duplex DNA processively is λ-exonuclease (21). These seemingly unique features of T5 DNA polymerase make it an extremely interesting model for the study of processive enzymes.

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