On the Association of Reverse Transcriptase with Polynucleotide Templates during Catalysis†

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ABSTRACT: The association of avian myeloblastosis virus (AMV) DNA polymerase with polynucleotide templates during catalysis has been studied. During the course of polymerization, different template-primer complexes were added and the ability of the enzyme to switch from one polynucleotide template to another was determined. At 37 °C as well as at 4 °C, the polymerase is able to switch from certain template-primer complexes to others. For example, the addition of poly(A)·oligo(dT) during the course of synthesis with poly(C)·oligo(dG) results in the immediate cessation of dGMP polymerization and the start of dTMP polymerization without any lag. Early during the course of polymerization, the size of the product, as determined by alkaline sucrose gradient centrifugation, is, in part, a function of the ratio of the template-primer complex to the enzyme. These cumulative experiments indicate that catalysis on polynucleotide templates with avian myeloblastosis virus DNA polymerase under the conditions tested is not processive in a classical sense. Similar to cellular DNA polymerases the enzyme can shift from one template-primer to another. Using autoradiography after gel electrophoresis to estimate the product size, it can be calculated that the enzyme switches from one template to another within 0.25 min at 37 °C which corresponds to the incorporation of >25 nucleotides. At 4 °C, switching can be calculated to occur in less than three nucleotide addition steps. Thus, with certain homopolymers, conditions can be found by which AMV DNA polymerase can switch from one template-primer complex to another, perhaps after each nucleotide addition step.

Until recently it has been assumed that DNA[1] polymerization in vivo and in vitro is carried out in a processive manner; i.e., once synthesis starts, the enzyme remains associated with the template until completion of the newly synthesized chain. Critical experiments bearing on this aspect of the mechanism of catalysis have recently been published. Polymer challenge experiments with *Escherichia coli* Pol I suggest that the mechanism is distributive; the enzyme dissociates readily from the enzyme-template complex after each catalytic step (McClure and Jovin, 1975). Chang (1975) has reported similar results with *E. coli* Pol I as well as DNA polymerases α and β from calf thymus. In contrast, studies with *E. coli* Pol I demonstrate that 12 nucleotides on the cohesive ends of λ DNA are copied without dissociation of the enzyme from the template suggesting a processive mechanism (Uyemura et al., 1975).

Studies with the DNA polymerase from avian myeloblastosis virus ("reverse transcriptases") offer advantages for this type of analysis. The enzyme can be obtained in homogeneous form (Grandgenett et al., 1973; Kacian and Spiegelman, 1974) and is devoid of any detectible exodeoxynuclease activity (Seal and Loeb, 1976). A comparison of the mechanism of catalysis between DNA polymerases from RNA tumor viruses and cellular DNA polymerase is needed to establish whether the mechanism for catalysis by viral polymerase is unique.

Materials and Methods

Plasma from chickens infected with avian myeloblastosis virus was a generous gift of W. J. Beard, Life Sciences Building, St. Petersburg, Fla. The plasma was stored at −70 °C prior to purification of the virus.

Unlabeled deoxynucleotides were purchased from Calbiochem. Tritium-labeled and α-^{32}P-labeled nucleotides were obtained from New England Nuclear. Polynucleotides having different sizes were obtained from P-L Biochemicals or were sized on the basis of sedimentation in sucrose gradients (Battula and Loeb, 1975). Poly[d(A-T)]·poly[d(A-T)] was prepared by a de novo catalyzed reaction using *E. coli* DNA polymerase I (Radding and Kornberg, 1962). In this paper the concentration of polymers is stated in terms of molecular weight and not nucleotide phosphorus. ^{3}H-labeled polyoma DNA (6-7 S) was a gift of Dr. J. Summers (The Institute for Cancer Research, Fox Chase, Philadelphia, Pa.) and 2′,3′-dideoxythymidine triphosphate was a gift of Dr. J. G. Moffatt (Syntex Research Institute, Palo Alto, Calif.).

Purification of AMV DNA Polymerase. Avian myeloblastosis virus was isolated by sedimentation and equilibrium centrifugation in sucrose gradients. The DNA polymerase was purified from the isolated virions as previously described (Kacian and Spiegelman, 1974; Battula and Loeb, 1974). The purest fraction of the holoenzyme obtained after phosphocellulose chromatography was further fractionated by gel filtration on Sephadex G-100. The polymerase was homogeneous as determined by isoelectric focusing in pH-stabilized glycerol gradients in that activity with the complementary and non-complementary nucleotide substrates (Battula and Loeb, 1974) and zinc content (Poiesz et al., 1974) were proportional to protein content across the peak. Upon electrophoresis in sodium dodecyl sulfate-polyacrylamide gels, the enzyme displays only two distinct protein bands of molecular weight 65 000 and

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[1] Abbreviations used: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; AMV, avian myeloblastosis virus; poly(A), poly(adenylic acid); oligo(dT), oligo(deoxythymidylic acid); poly(C), poly(cytidylic acid); oligo(dG), oligo(deoxyguanylic acid); poly(rA), polyriboadenylate; dGMP, deoxyguanosine monophosphate; poly(rC), polyribocytidylate; dTMP, deoxythymidine monophosphate; dTTP, deoxythymidine triphosphate; dGTP, deoxyguanosine triphosphate; poly(A-T), poly(adenylthymidine); dATP, deoxyadenosine triphosphate; EDTA, ethylenediaminetetraacetic acid; Tris,tris(hydroxymethyl)aminomethane.

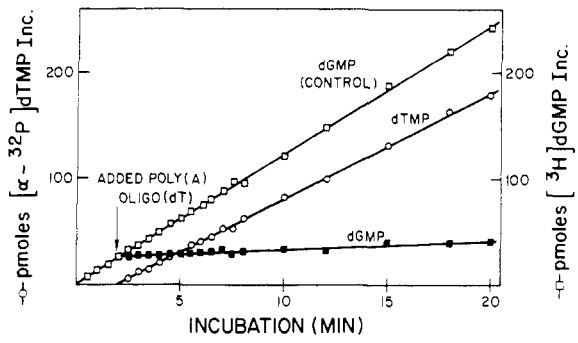

FIGURE 1: Effect of the addition of poly(A)·oligo(dT) on poly(C)·oligo(dG) directed synthesis by AMV DNA polymerase. The reaction mixture in a total volume of 0.5 ml contained 3.0 nmol of $(pC)_{2500}$·$d(pG)_{12-18}$ (1:1), 40 μM [^{3}H]dTTP (500 cpm/pmol), 40 μM [α-P^{32}]dGTP (100 cpm/pmol), 50 pmol of AMV DNA polymerase, and others constituents as given under Materials and Methods. After 2 min of incubation at 37 °C, 6.0 nmol of $(pA)_{500}$·$d(pT)_{12-18}$ (1:1) was added to the incubation mixture, 20-μl aliquots were removed at the indicated times, and the radioactivity in acid-insoluble product was determined. Control reactions each containing only a single template-primer complex were analyzed at the same time.

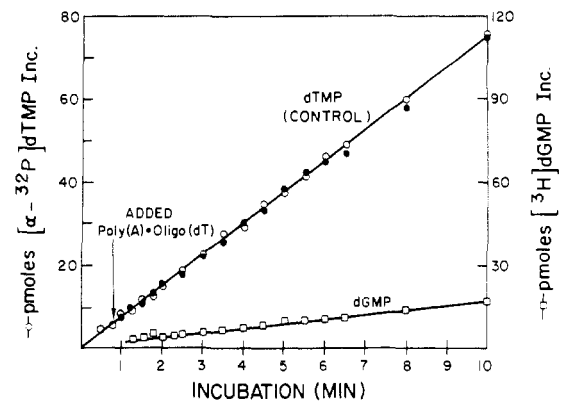

FIGURE 2: Effect of the addition of poly(C)·oligo(dG) on poly(A)·oligo(dT)-directed synthesis. The standard incubation mixture in a total volume of 0.5 ml contained 0.4 nmol of $(pA)_{3000}$·$d(pT)_{12-18}$ (1:1), 40 μM [^{3}H]dGTP (500 cpm/pmol), 40 μM [α-P^{32}]dTTP (200 cpm/pmol), and 50 pmol of AMV DNA polymerase. After 1 min of incubation at 37 °C, 4000 pmol of $(pC)_{300}$·$d(pG)_{12-18}$ (1:1) was added. Radioactivity in the acid-insoluble precipitate was determined as in Figure 1. The open circles indicate incorporation of [α-^{32}P]dTMP with poly(A)·oligo(dT) as template and the closed circles dTMP incorporation after the addition of poly(C)·oligo(dG).

110 000 which have been designated by others (Grandgenett et al., 1973) as the α and β subunits, respectively.

Polymer Challenge Experiments. The standard assay contained the following in a total volume of 0.5 ml: 40 μM of two labeled radioactive deoxyribonucleoside triphosphates (one labeled with ^{3}H and the other with α-^{32}P); 50 mM Tris-HCl, pH 8.0; 5 mM dithiothreitol; 20 mM KCl; 8 mM $MgCl_2$; 20 μg of bovine serum albumin; varying amounts of a template-primer complex and AMV DNA polymerase. The assays were incubated at 37 °C for 2 to 4 min and then a second template-primer or buffer alone was added. Aliquots (0.01 or 0.02 ml) were removed at different times, and incorporation of the radioactive nucleotides into acid-insoluble precipitate was determined after repeatedly precipitating the product with 1.0 N perchloric acid and solubilizing in 0.2 N NaOH (Battula and Loeb, 1974).

Results

Effect of Adding a Second Template-Primer Complex during Polymerization. In order to study the association of AMV DNA polymerase with template during catalysis, we asked whether the polymerase could shift from one to another template-primer complex prior to completion of the first chain. The initial reaction mixture contained 50 pmol of enzyme, 3.0 nmol of $poly(C)_{2500}$·$oligo(dG)_{12-18}$[2] (1:1) (based on molecular weight and not nucleotide phosphorus), and [α-P^{32}]dTTP as well as [H^3]dGTP. Thus for each molecule of enzyme there was present 60 molecules of the template-primer complex. As expected only dGMP was incorporated into an acid-insoluble product (Figure 1, dGMP control). After 2 min of synthesis, 6.0 nmol of poly(A)·oligo(dT) was added to the reaction. The results in Figure 1 show that further dGMP incorporation was immediately stopped. Thereupon incorporation of [α-P^{32}]dTMP commenced. This observation indicates that AMV DNA polymerase can switch from the first template-primer complex, poly(C)·oligo(dG), prior to completion of the entire chain and initiate synthesis on the added second template-primer complex, poly(A)·oligo(dT). It is important to note that no lag was observable and that the rate of dTMP incorporation after the addition of the second template was almost identical with that of control reaction mixtures in which only poly(A)·oligo(dT) template was present (data not shown). In addition, no lag was observed in experiments in which both the first and second template-primers were present in equal concentrations.

The converse experiment was also carried out (Figure 2). In the initial reaction, 50 pmol of enzyme was present, 0.4 nmol of poly(A)·oligo(dT) was the initial template-primer, and 4.0 nmol of poly(C)·oligo(dG) was used as the second template-primer. In contrast to the first observation, a tenfold greater amount of the second template did not significantly inhibit incorporation on the first template. Furthermore, the rate of incorporation of dGMP on the second template was approximately one-tenth that of control tubes in which only the second template was added (data not shown). The lack of switching was also found at a low nucleoside triphosphate concentration of 2 μM and also with poly(C)·poly(dG) as the second template-primer.

In order to account for the lack of switching of AMV DNA polymerase from poly(A)·oligo(dT) upon addition of the second template, poly(C)·oligo(dG), we measured nucleotide incorporation as a function of template concentration. In experiments under conditions identical with those previously used (Figures 1 and 2) half-saturation was achieved with 40 to 50 nM for poly(A)·oligo(dT) and 320 to 350 nM for poly(C)·oligo(dG) (data not shown). The enzyme concentration was 40 nM and the complementary nucleotide was 49 μM. V_{max} for dTMP and dGMP was 4.0 and 6.0 nucleotides/min with the respective complementary templates. These results suggest that rates of synthesis with both templates are similar but that poly(A)·oligo(dT) binds more tightly with the enzyme. To approximate the situation in the template challenge experiments, the concentration of the two template-primers were varied in the same reaction (Figure 3). In these experiments, the amount of template was kept constant but the ratio of poly(A)·oligo(dT) to poly(C)·oligo(dG) was varied. Again, both [α-^{32}P]dTTP and [^{3}H]dGTP were present in each reaction mixture. It can be extrapolated that poly(C) is copied with an efficiency equal to that of poly(A) only if it is present in a 20-fold greater excess than poly(A). This result is in reasonable accord with the six- to nine-fold greater K_m for poly(C) than

[2] Subscripts for polynucleotides indicate the average residue length.

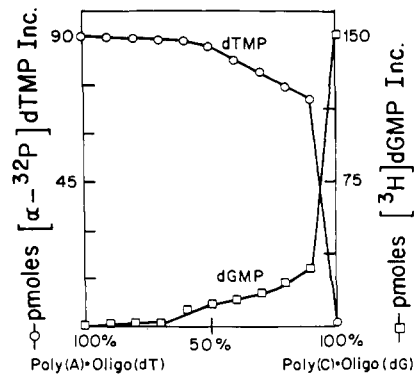

FIGURE 3: Competition reactions between different template-primers. In these reactions the ratios of $(pA)_{500}$•$d(pT)_{12-18}$ (1:1) to $(pC)_{300}$•$d(pG)_{12-18}$ (1:1) were varied. The reaction mixture in a volume of 25 μl contained a total of 2 μg of the template-primer, 40 μM [^{3}H]dGTP (100 cpm/pmol), 40 μM [α-^{32}P]dTTP (20 cpm/pmol), 2 pmol of AMV DNA polymerase, and other constituents in the same concentrations as in the standard reaction mixture. On a molar basis, 100% poly(C)•oligo(dG) corresponds to 800 nM and 100% poly(A)•oligo(dT) corresponds to 440 nM. Incubation was carried out at 37 °C for 15 min.

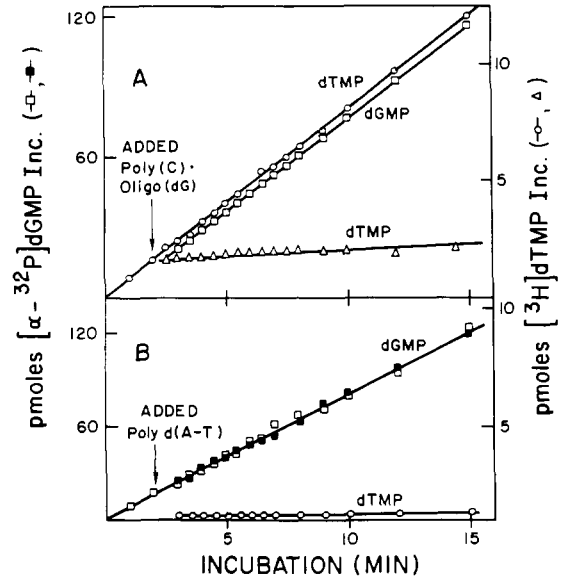

FIGURE 4: (A) Effect of poly(C)•oligo(dG) on polyd(A-T)-directed synthesis. The standard reaction mixture in a total volume of 0.5 ml contained 6 nmol of poly[d(A-T)], 40 μM [α-^{32}P]dGTP (20 cpm/pmol), 40 μM [^{3}H]dTTP (500 cpm,pmol), 100 μM dATP, and 50 pmol of AMV DNA polymerase. After 2 min of incubation at 37 °C, 8 nmol of $(pC)_{300}$•$d(pG)_{12-18}$ was added to the reaction mixture. Aliquots of 15 μl were removed at the indicated times. In B, the order of addition of the two templates was reversed. The initial template-primer was 6 nmol of $(pC)_{3000}$•$d(pG)_{12-18}$ and 13 nmol of polyd(A-T) was added at 2 min.

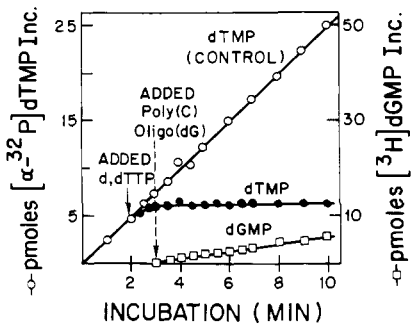

FIGURE 5: Effect of termination by 2′,3′-dideoxy-TTP. The standard reaction mixture in a total volume of 0.5 ml contained 50 pmol of AMV DNA polymerase, 0.15 nmol of $(pA)_{500}$•$d(pT)_{12-18}$ (1:1), 20 μM [α-P^{32}]dTTP (200 cpm/mol), and 40 μM [^{3}H]dGTP (500 cpm/pmol). After 2 and 4 min of incubation, respectively 60 μM dideoxy-TTP and 3 nmol of $(pC)_{300}$•$d(pG)_{12-18}$ (1:1) were added. Equivalent volumes of buffer were added to the control tube.

for poly(A) and may account for the inability of poly(C)•oligo(dG) to be utilized immediately for incorporation in the polymer challenge with poly(A)•oligo(dT) as the initial template-primer. Thus, in this case, if the enzyme dissociates from the first template during catalysis it is more likely to reassociate with that template than a different template to which it binds less tightly.

Competition between Ribonucleotide and Deoxyribonucleotide Template. Polymer-challenge experiments were carried out using poly(C)•oligo(dG) and poly[d(A-T)] as templates (Figure 4A). As expected, the rate of synthesis with polyd(A-T) as a template was lower than that obtained with polyribonucleotide templates. Upon addition of the second template [poly(C)•oligo(dG)], polymerization on the initial template polyd(A-T) was entirely terminated. The ratio of the second template to the first was 1:1.3. The rate of incorporation upon the second template was nearly as great as that in control tubes with only the second template (results not shown). If the reaction is initiated with poly(C)•oligo(dG), the addition of twofold greater amount of polyd(A-T) is without effect (Figure 4B). These results suggest that poly(C)•oligo(dG) has a greater affinity in binding AMV DNA polymerase than does polyd(A-T).

Effect of Termination on Polymer-Challenge Experiments. The effect of terminating synthesis upon the association of the enzyme with the template is shown in Figure 5. In this experiment poly(A)•oligo(dT) was the initial template and 2′,3′-dideoxythymidine triphosphate was added to the reaction after polymerization proceeded for 2 min. Thereupon, incorporation of dTMP was abruptly terminated. After termination, the addition of a second template, poly(C)•oligo(dG), did not result in the incorporation of its complementary nucleotide, dGMP. Control experiments indicate that 60 μM dideoxythymidine triphosphate has no effect on dGMP incorporation with poly(C)•oligo(dG) as template-primer. Thus termination of synthesis by itself is insufficient to dissociate the enzyme from the template.

In order to show that the enzyme can be dissociated from a template after termination, we carried out the reaction in a large excess of the template-primer complex (Figure 6). In this experiment the ratio of initiated poly(A)•oligo(dT) to enzyme was approximately 320:1. Synthesis was terminated after 2 min by the addition of 2′,3′-dideoxythymidine triphosphate. If 2′,3′-dideoxythymidine triphosphate was utilized at a rate as great as the substrate, dTTP, incorporation of dideoxythymidine monophosphate would be sufficient to terminate about 40% of the added molecules of templates. Upon the addition of 1 mM thymidine triphosphate (tenfold greater than the concentration of dideoxythymidine triphosphate) polymerization on poly(A)•oligo(dT) was resumed. These results suggest that the enzyme stays bound to template-primer complexes without 3′-OH termini and can shift from such a terminated primer to start synthesis on new template-primer complex.

Inhibition of Polymerization Using Added Unprimed Templates. Since the enzyme binds to the template-primer without a 3′-OH primer terminus, it is possible that the shift from one template-primer complex to another might not require an added primer. The results in Figure 7A indicate that the addition of a large amount of poly(A) [20-fold greater than

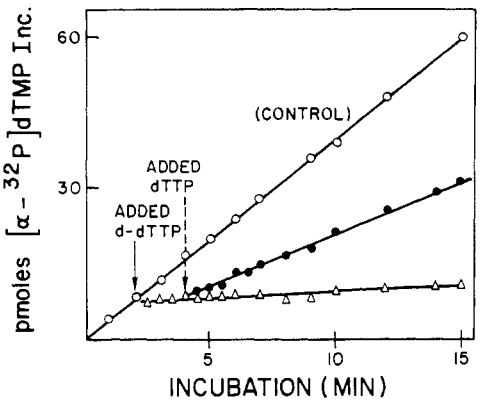

FIGURE 6: Effect of the sequential additions of dideoxy-TTP and dTTP on poly(A)·oligo(dT) (1:1) directed poly(dT) synthesis. The reaction mixture in a total volume of 0.5 ml contained 16 nmol of $(pA)_{500}$·$d(pT)_{12-18}$ (1:1), 20 μM [α-P^{32}]dTTP (50 cpm/pmol), and 50 pmol of enzyme. After 2 min of incubation dideoxy-TTP was added to a concentration of 100 μM, and after 4 min of incubation [α-P^{32}]dTTP (50 cpm/pmol) was added to a final concentration of 1 mM. To the control tube, an equal volume of buffer was added each time.

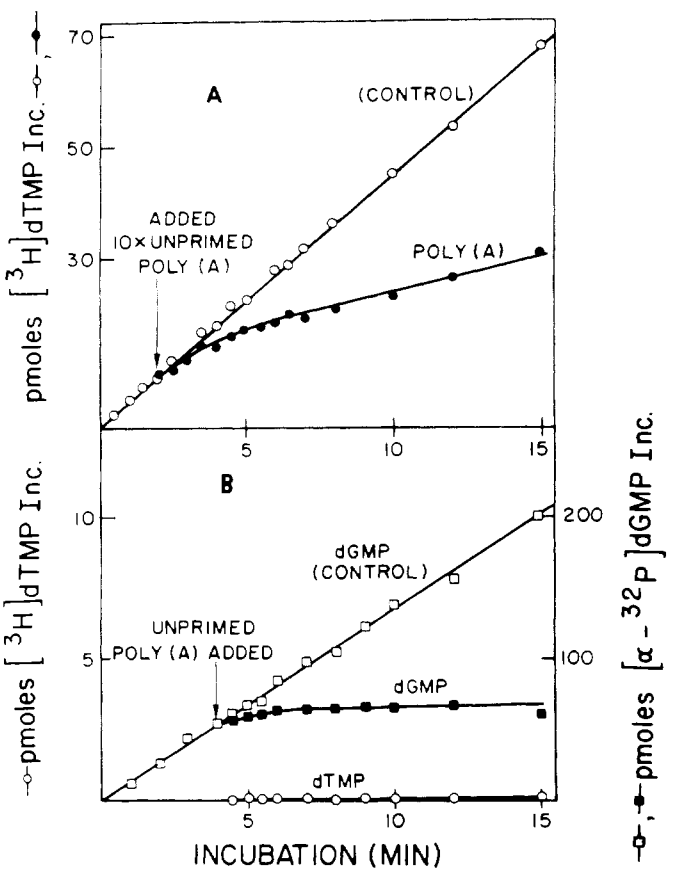

FIGURE 7: Effect of unprimed template. The initial mixture is the same as that in Figure 2, except that [^{3}H]dTTP was the only nucleotide present. After 2 min of incubation, 8 nmol of $poly(A)_{500}$ was added. In Figure 7B the initial reaction was the same as in Figure 1. After 4 min of incubation, 8 nmol of $poly(A)_{500}$ was added.

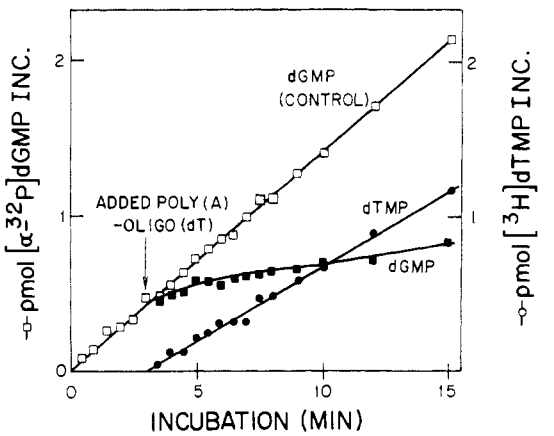

FIGURE 8: Effect of the addition of poly(A)·oligo(dT) on $poly(C)_{1100}$·$oligo(dG)_{12-18}$ directed synthesis at 4 °C. The reaction conditions are the same as those given in the legend to Figure 1.

the initial poly(A)·oligo(dT) complex] results in 50% inhibition in the rate of dTMP incorporation. Figure 7B shows a similar analysis using poly(C)·oligo(dG) as the initial template-primer complex. After synthesis for 2 min the addition of a tenfold excess of poly(A) markedly inhibits the dGMP incorporation. As seen, the added poly(A) without a primer does not stimulate incorporation of dTMP. The inhibition observed could result from the dissociation of the enzyme from the actively synthesizing complex and reassociation with the unprimed template. The latter, lacking a proper primer terminus, is not adequate for polymerization (Gillespie et al., 1975).

Polymer Challenge Experiments at 4 °C. Following the suggestion of McClure and Jovin (1975) polymer challenge experiments were carried out at 4 °C. The rate of nucleotide polymerization is reduced so that one can determine whether the enzyme can be removed from each template within a few nucleotide addition steps. The initial reaction contained 50 pmol of AMV DNA polymerase and 3.0 nmol of poly(C)·oligo(dG). The rate of synthesis at 4 °C is about 1/90 of that obtained at 37 °C (compare Figures 1 and 8). After 2 min of incubation, 6.0 nmol of poly(A)·oligo(dT) were added, incorporation of dGMP onto the initial template was strikingly inhibited, and the incorporation of dTMP was immediately initiated. This change from one template to a different template was observed in less than 1 min (Figure 8).

Relationship between Size of Product and the Ratio of Template to Enzyme. Previous studies from this laboratory indicated that, in the presence of excess template-primer, the size of the product was unimodal and increased with time (Battula and Loeb, 1975). This suggested that AMV DNA polymerase could have been removed from each template during growth. However, in those experiments no evidence was obtained for small newly synthesized polynucleotides even in experiments carried out for only 3 min at 20 °C. A more detailed analysis of the relationship of size of the product to template-primer concentration is shown in Figure 9. The upper graph represents an alkaline sucrose gradient centrifugation of the product synthesized in a reaction containing 12 pmol of enzyme and 16 pmol of the template-primer complex incubated at 6 min at 37 °C (Figure 1). The lower graph represents a similar analysis except that the template concentration in the reaction was tenfold greater. As observed the extent of synthesis measured by the total area is greater in the lower graph since nearly saturating amounts of the template were present. However, the size of the product is greatly reduced. Similar changes in the sedimentation pattern of the polynucleotide product were observed when poly(C)·oligo(dG) was used as template-primer. Even though the size of the product is not reduced in exact proportion to the difference in the ratio of enzyme to template (as one might expect from a random distributive process), the extent of reduction (two- to fourfold) strongly suggests that the enzyme is able to shift from one template to another during the course of the reaction.

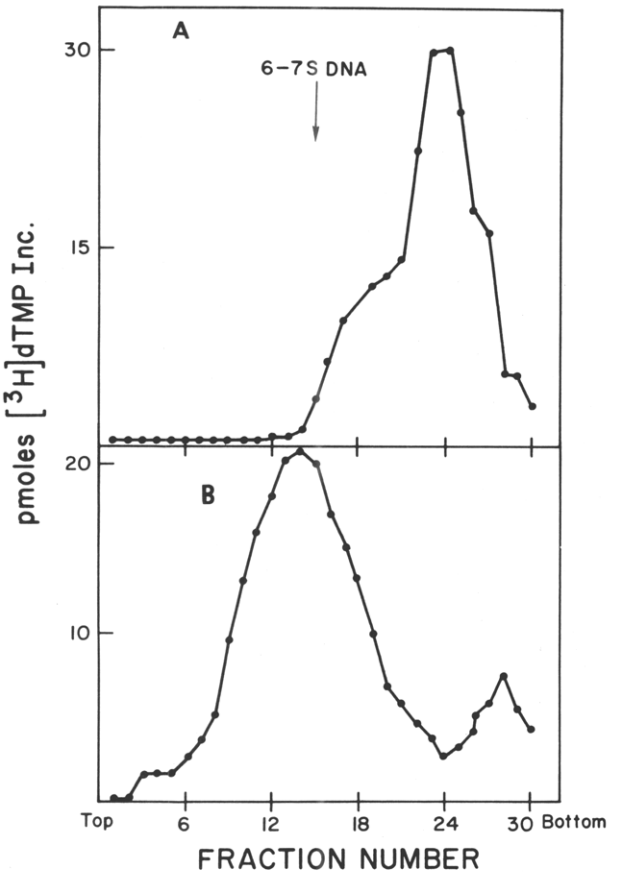

FIGURE 9: Size of poly(dT) synthesized at two different concentrations of poly[r(pA)]$_{3000}$·poly[d(pT)]$_{12-18}$ (1:1). (A) The reaction mixture in a total volume of 0.15 ml contained 16 pmol of poly(pA)$_{3000}$·d(pT)]$_{12-18}$, 40 μM [^{3}H]dTTP (200 cpm/pmol), 12 pmol of AMV DNA polymerase, and other constituents in the same concentrations as in the standard reaction mixture. After incubation at 37 °C for 6 min, the reaction was stopped by the addition of EDTA (to a final concentration of 10 mM). (B) The reaction mixture is the same as in A, except that a tenfold greater concentration of the template–primer was present. For each gradient 100 μl of each reaction mixture was layered directly over a linear 5 to 20% sucrose gradient (5 ml) containing 0.1 M NaOH, 0.1 M NaCl, and 0.2% sarkosyl. The gradients were centrifuged in a Spinco SW 50.1 rotor at 45 000 rpm for 12 hr at 20 °C. Fractions were collected from the top of the gradients and radioactivity was determined after adding 100 μg of calf-thymus DNA precipitating with 1.0 M perchloric acid and pouring onto glass fiber filters. The arrow indicates the position of 6–7S fragments of ^{3}H-labeled polyoma DNA digested with *Hemophilus aegyptius* endonuclease R·Hae III (Summers, 1975).

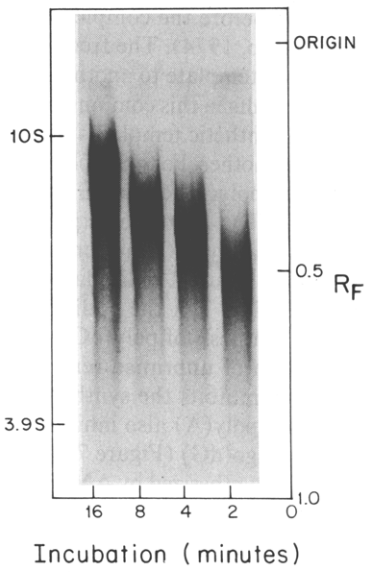

FIGURE 10: Autoradiogram of agarose–polyacrylamide gel separation of the products synthesized by AMV DNA polymerase. The reaction mixture in a total volume of 100 μl contained the following: 100 μM [α-^{32}P]dTMP (2000 dpm/pmol); 50 mM Tris-HCl (pH 7.6); 5 mM $MgCl_2$; 2 pmol of poly(A)·oligo(dT)$_{12-18}$; and 2 pmol of AMV DNA polymerase. The poly(A) template was 10 S as determined by sedimentation in sucrose gradients. The reaction was stopped by the addition of 10 mM EDTA at different time intervals. The reaction mixtures were then made 0.5% sodium dodecyl sulfate and 0.1 M KOH and incubated for 30 min at 90 °C. Aliquots after hydrolysis containing 5000 dpm of an acid-insoluble product were mixed with sucrose and applied to 2% polyacrylamide slab gels containing 0.5% agarose. Electrophoresis was carried out for 3 h at 75 V according to the method described by Summers (1975). In a parallel experiment, poly(C) polymers of different sizes were used as markers. The poly(C) polymers were sized by analytical centrifugation. After electrophoresis, the gels were stained with methylene blue (Peacock and Dingman, 1967). The product synthesized on 10S poly(A) corresponds to approximately 10 S as determined by alkaline sucrose gradient (Kacian and Spiegelman, 1974) and has approximately the same electrophoretic mobility as 10S poly(C). Assuming the molecular weight of 10 S poly(dT) is approximately equal to 4.4×10^5 (Studier, 1965), the length of the product (16 min) corresponds to approximately 1250 nucleotides.

Rate of Chain Elongation. One can estimate minimum rate of chain elongation in the polymer challenge experiments by making several assumptions (Figure 1). If the mode of catalysis is strictly processive, all protein added is active enzyme, and each enzyme forms an active complex, then chain elongation with poly(A) as a template is equivalent to 5 mol of nucleotide per mol of enzyme per min at 37 °C. At the other extreme, if the mode of catalysis is solely distributive and each primer is utilized then chain elongation is 0.1 mol of nucleotide per mol of primer per min at 37 °C. However, the rate of chain elongation as estimated by alkaline sucrose gradient analysis is much greater than either of these estimates. We find that the predominant size of the product synthesized for 6 min at 37 °C corresponds to an *s* value greater than 6–7, suggesting molecules synthesized several hundred nucleotides in length.

In order to obtain an estimate of the rate of chain elongation, the electrophoretic mobility of product synthesized for increasing lengths of time was determined. In these experiments, the molar ratio of AMV DNA polymerase to poly(A)·oligo(dT) was 1:1 and the newly synthesized product was identified by autoradiography after alkaline hydrolysis and electrophoresis in polyacrylamide–agarose gels (Figure 10). The molecular weight of each product was estimated from the relative mobility (Loening, 1969). The size of the product increased linearly with time of incubation; by 16 min at 37 °C it corresponds in electrophoretic mobility to a marker of poly(A) of 10 S. Assuming a molecule of poly(dT) of 10 S corresponds to 1250 nucleotides in length (Studier, 1965), then the rate of chain elongation was about 80 nucleotides per min at 37 °C.

Discussion

During chain propagation, DNA polymerase can be postulated to behave in either of the following ways: (1) the enzyme may be processive (i.e., once bound to a template, the enzyme completes the replication of that template before dissociating and copying a second template); or (ii) the enzyme may be distributive (i.e., the enzyme dissociates from the template after each nucleotide addition step).

Previous results from our laboratory on size distribution of the products using AMV DNA polymerase copying synthetic polynucleotides suggested that the enzyme may leave a tem-

plate–primer complex before the complexation of the entire chain (Battula and Loeb, 1974). The frequency of switching from one homopolymer template to another was not apparent in those studies. The results in this communication suggest that (i) while copying one synthetic template–primer complex, the enzyme may switch to another. For example, AMV polymerase may be removed from poly(C)·oligo(dG) by the addition of excess poly(A)·oligo(dT). In these experiments poly(dT) synthesis starts immediately with the concomitant inhibition of poly(dG) synthesis (Figure 1). Similarly, while synthesis is proceeding on poly[d(A-T)], the addition of poly(C)·oligo(dG) initiates the synthesis of poly(dG). (ii) During polymerization the addition of unprimed templates inhibits the reaction; e.g., poly(A) inhibits the synthesis of poly(dT) on poly(A)·oligo(dT) and poly(A) also inhibits the synthesis of poly(dG) on poly(C)·oligo(dG) (Figure 7). (iii) Furthermore, the size of the poly(dT) synthesized by AMV DNA polymerase has been found to be diminished considerably in the presence of excess poly(A)·oligo(dT) (Figure 9).

Our results suggest that AMV DNA polymerase when copying synthetic polynucleotides can switch from one template to the other before completion of the entire chain and perhaps during each catalytic event. Similar conclusions have been reported for cellular DNA polymerases. Experiments of McClure and Jovin (1975) indicate that *E. coli* Pol I within one catalytic step can switch from polyd(A-T) to activated DNA. In these experiments, the ratio of template to polymerase was nearly equal and thus free enzyme could have been present in the reaction mixture. Chang (1975) reported that *E. coli* DNA Pol I as well as the large and small DNA polymerases from calf thymus are distributive when copying homopolymers at 37 °C. At this temperature the rate of chain elongation is such that hundreds of nucleotides could be polymerized during the time the enzyme is observed to switch from one template to another. In contrast, when copying the cohesive ends of λ DNA, *E. coli* DNA Pol I remained associated with the template until the entire 12 nucleotide termini were completed, thus establishing that polymerization can be processive in at least this unique situation (Uyemura et al., 1975).

A gross estimation of the size of the product of the reaction can be obtained from electrophoretic mobility on polyacrylamide gels. Since both poly(C) and poly(dT) have been reported to exist as single-stranded polymers at neutral pH (Riley et al., 1966; Fasman et. al., 1964), sized poly(C) molecules were used as markers. With AMV DNA polymerase, the rate of chain elongation, as determined by the electrophoretic mobility of the product, corresponds to 75–125 nucleotides/min at 37 °C. Since the observed changes from one template to another occur in less than 0.5 min, it is possible that many nucleotides are polymerized prior to dissociation of the polymerase from the template. The 90-fold decrease in rate of incorporation at 4 °C presumably represents a proportional decrease in the rate of elongation. If so, the observed shift from one template to another occurred within one to three nucleotide addition steps. This estimate of 100 nucleotides/min at 37 °C is 20-fold greater than estimates based on incorporation per molecule of added enzyme protein (Figure 1). Conditions for synthesis in both systems are not exactly comparable. The rate of chain growth as determined by nucleotide incorporation (Figure 1) assumes that all enzyme molecules are active and that each enzyme molecule catalyzes polymerization at one growing point. The analysis of chain elongation by electrophoresis is direct and requires none of these assumptions.

It is to be noted that poly(rC)·oligo(dG) was incapable of removing the enzyme from poly(rA)·oligo(dT) (Figure 2). Competition experiments suggest that the enzyme has a 20-fold greater affinity for poly(rA)·oligo(dT) complex than for poly(rC)·oligo(dG) (Figure 3). Poly(A) has been found to be present at the 3′ end of the cellular and viral mRNA (Gillespie et al., 1972). Inhibition of poly(dT) synthesis by cordycepin has been found to inhibit AMV replication (Richardson et al., 1975), suggesting that the poly(rA) tracts are needed for replication of the virus. If this is so, the high affinity for poly(rA) might serve to position the viral DNA polymerases prior to DNA synthesis. However, in vitro studies indicate that DNA synthesis starts from a unique site of the genome near its 5′ terminus (Taylor and Illmensee, 1975). In addition there is evidence that the poly(A) sequence at the 3′ terminus does not function as a primer binding site (Reitz et al., 1972).

The requirements for synthesis with AMV DNA polymerase are similar if not identical with those of other cellular DNA polymerases (Loeb, 1974). AMV DNA polymerase like other DNA polymerase (Slater et al., 1971) has been shown to contain stoichiometric amounts of zinc (Auld et al., 1974; Poiesz et al., 1974) and the zinc appears to be present on the catalytic active α subunit (Poiesz et al., 1974). Like the DNA polymerases from animal cells (Slater et al., 1971), AMV DNA polymerase lacks any detectable 3′ → 5′ exodeoxynuclease activity (Battula and Loeb, 1976). However, the DNA polymerase from RNA tumor viruses has been reported to contain an RNase H activity (Molling et al., 1971; Baltimore et al., 1974) which hydrolyze exonucleolytically the RNA strand of a DNA–RNA hybrid. Studies of Grandgenett and Green (1974) indicate that with the holoenzyme (α and β subunit) the RNase H activity of the polymerase degrades a polynucleotide completely prior to initiating hydrolysis on a second chain, suggesting that the hydrolysis is processive. In contrast, hydrolysis with the isolated α subunit follows a distributive mode. The studies in this paper were carried out with samples of purified AMV DNA polymerase in which the α and β subunit were in equal proportions. Our results on a lack of processivity for polymerization are not necessarily in disagreement with those on RNase H activity. First, the conditions of assay for measuring RNase H activity could have permitted the entire template to be copied prior to the initiation of hydrolysis by RNase H. Secondly, the sites on the enzyme for polymerization and RNase H are probably different and thus the association of the enzyme with the template may be dependent on which reaction is being catalyzed. Also, it should be noted that there are no studies that vigorously establish a physical dissociation of the polymerase from a template during catalysis.

The question of whether an enzyme is processive or distributive is of mechanistic importance but may have little bearing on the realities of DNA replication. It is to be noted that most experiments apply only to synthetic polynucleotides under in vitro conditions. Within virions the dissociation of the enzyme from the template during catalysis could be minimized by physical boundaries or by other proteins that affect the affinity of the polymerase for the template. In bacteria it is firmly established that multiple proteins participate concertedly in DNA replication (Alberts, 1973). Furthermore the conditions in the test tube which permit studies in the presence of a high multiplicity of competing template and primer molecules are obviously not pertinent to the structural constraints during DNA replication in eucaryotic cells and even in procaryotic cells. In vivo replication of DNA proceeds on a structured environment where there is little chance for competition between different chromosomal components.

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