Processive Nature of Reverse Transcription by Avian Myeloblastosis Virus Deoxyribonucleic Acid Polymerase†

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ABSTRACT: The ribonucleic acid dependent deoxyribonucleic acid polymerase from avian myeloblastosis virus was shown to synthesize poly(dT) transcripts by a processive mechanism using poly(rA)$_{1100}$·oligo(dT)$_{12-18}$ as template and primer. Template challenge experiments demonstrate that at low temperature and ionic strength, the polymerase remains bound to the completed template·daughter strand complex after completion of daughter strand elongation. Higher temperatures and ionic strength increase the dissociation of the enzyme from the complex, thus reducing transcript length. Analysis of product size and quantity indicates that the degree of processivity is also influenced by the types and concentrations of metal ions present and indicates that the metal ions affect the activity of the poly(rA) as a template more than they affect processivity or enzyme activity. The results also lead to the conclusion that initiation is the rate-limiting step under all of our experimental conditions. The arguments for a processive as opposed to distributive mechanism are based on an analysis of enzyme–template interactions, product size, and amount of product made under specific reaction conditions.

Two possible mechanisms of enzyme–template interaction during chain elongation by retrovirus DNA polymerases have been postulated. The distributive hypothesis states that the polymerase dissociates from the template·primer complex after each addition of a nucleotide to the growing daughter strand, while the processive hypothesis proposes that the enzyme remains associated with the same template and daughter strand through many nucleotide additions. The degree of processivity could range from the incorporation of only a few to thousands of nucleotides before enzyme dissociation. If a polymerase was processive, it should be expected that the degree of processivity would be influenced by the reaction conditions. Two different reports have used kinetic evidence in attempts to answer the question of whether or not retrovirus DNA polymerase is processive (Leis, 1976) or distributive (Dube & Loeb, 1976), and both conflicting conclusions have been reached based on similar kinetic experiments. Conflicting results have also been reported for *Escherichia coli* DNA polymerase I (Uyemura et al., 1975; Das & Fujimura, 1979; Bambara et al., 1978; Chang, 1975; McClure & Jovin, 1975; Sherman & Gefter, 1976). Using a quantitative analysis of the product size distribution together with kinetic data, we demonstrate that transcription by the RNA-dependent DNA polymerase from avian myeloblastosis virus (AMV)[1] is processive on the poly(rA)·oligo(dT) template·primer complex. Evidence is also presented which demonstrates that initiation is the rate-limiting step in the in vitro reactions, and a lower limit for the elongation rate is set. The effects of reaction conditions on the degree of processivity are also examined. The rationale and techniques described in this paper should also be applicable to other polymerases with appropriate choices of template and primer.

Materials and Methods

AMV DNA Polymerase. Purified AMV reverse transcriptase was obtained from J. Beard through NIH Program Resources and Logistics. Density gradient analysis of the enzyme showed a single, symmetrical peak of activity with a molecular weight of 160000 corresponding to the holoenzyme. No activity corresponding to free α subunits was found.

Template, Primer, and Nucleotides. Poly(rA) and oligo(dT)$_{12-18}$ were purchased from P-L Biochemicals. The poly(rA) was sized by us to an average length of 1100 nucleotides [poly(rA)$_{1100}$] by using high-pressure liquid chromatography as described below. [*methyl*-^{3}H]Thymidine triphosphate was purchased from Amersham/Searle (sp act. 41 Ci/mmol) and diluted with unlabeled dTTP when necessary.

Polymerase Reactions. A variety of reaction conditions were used, and these details are presented where appropriate. Reactions were sampled by removing triplicate 10-μL aliquots which were quenched with 50 μL each of unlabeled 2 mM dTTP, 100 mM EDTA, 1 mg/mL BSA, and 1 mg/mL calf thymus DNA. Two hundred microliters of 10% Cl_3AcOH was added, and the precipitates were collected on glass fiber filters for counting.

High-Pressure Liquid Chromatography (LC). The use of LC has been described previously in our laboratory (Darling et al., 1977). The column consists of a 1-mL sample loop and injection valve, a 282 × 0.64 cm column packed with CPG-350 glycophase beads connected to a 305 × 0.64 cm column packed with CPG-715 glycophase beads, a UV detector, and a fraction collector. The sample injection loop was immersed in a boiling water bath to denature double-stranded nucleic acids. The LC column was immersed in a 75 °C water bath, and 0.1 M Tris-HCl, pH 7.2, was the eluant. One-milliliter fractions were collected beginning 20 min after sample injection at a flow rate of 100 mL/h. The DNA fragments used to calibrate the LC column were terminally labeled with ^{32}P (Maniatis et al., 1975) and detected by scintillation counting while the poly(rA), yeast tRNA, and dTTP were detected spectrophotometrically. Aliquots at 2 mg/mL were passed through the column to size the poly(rA). Poly(rA) which eluted in fractions 21–23 [poly(rA)$_{1100}$] was pooled and concentrated for

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[1] Abbreviations used: AMV, avian myeloblastosis virus; dTTP, deoxythymidine triphosphate; EDTA, ethylenediaminetetraacetic acid; BSA, bovine serum albumin; LC, high-pressure liquid chromatography; DTT, dithiothreitol; poly(rA), poly(riboadenylate); oligo(dT), oligo(deoxythymidylate); Cl_3AcOH, trichloroacetic acid; poly(dT), poly(deoxythymidylate).

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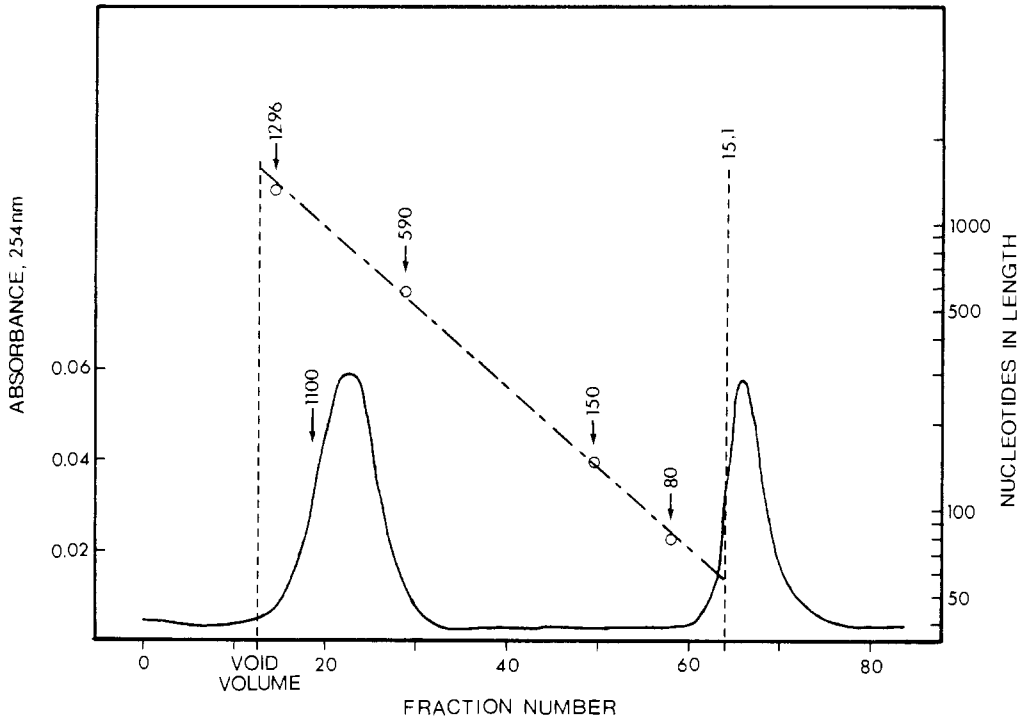

FIGURE 1: Elution profile of a sample of the sized poly(rA)$_{1100}$ and dTTP. Since this is an exclusion column, the volume corresponding to the half-maximal value of the leading edge of the peak is chosen as the elution volume of the sample. The positions of the markers indicated by arrows were found in previous runs. The 1296 nucleotide long fragment of SV-40 DNA and the 150- and 590-nucleotide fragments from a *Hin*dIII digest of λ DNA are also shown (the 1900-nucleotide and larger fragments eluted in the void volume with blue dextran). Oligo(dT)$_{12-18}$ did not separate from the dTTP and eluted in the included volume.

use in the assays. The polymerase reactions were terminated by immersion in a boiling water bath for 3 min, quickly cooled, and kept on ice. One-half of each reaction mixture was assayed for Cl_3AcOH-precipitable counts to determine the incorporation while the other half was applied to the LC column. An aliquot of each column fraction was counted to detect the ^3H-labeled poly(dT) products.

Results

Calibration and Use of the LC Column to Prepare the Poly(rA) Template. Figure 1 illustrates the elution profile of the poly(rA)$_{1100}$ and the position of several other markers used for calibration. The elution is linear over a range of 55–1600 nucleotides; shorter or longer pieces elute in the included and void volumes, respectively. The same column was used to analyze the sizes of the poly(dT) products of the polymerase reactions utilizing the poly(rA)$_{1100}$. Poly(rA) of this size was chosen so that the full-length transcripts obtained when the oligo(dT) primer hybridized at the 3′ end of the poly(rA) would not appear in the void volume.

Stable Enzyme·Template Complexes Formed during Transcription. The initial experiments examine the kinetics of poly(dT) synthesis under conditions designed to reveal the roles of ionic strength, template·primer concentration, and temperature on the association of the polymerase with the template and primer or daughter strand. The complex nature of these interactions is established by results which show that the reaction rate in the first 60 min of incubation is directly proportional to enzyme concentration but is not proportional to the poly(rA)·oligo(dT) concentration, demonstrating that the template·primer complex is not acting as a "substrate" in the classical kinetic scense (data not shown). At least three factors may contribute to this phenomenon. Firstly, since the ability of AMV polymerase to bind ribopolymers is well-known and the poly(rA) template used in these experiments was 1100 nucleotides in length, the concentration of enzyme binding sites on the poly(rA)$_{1100}$ is approximately 1100-fold greater than the concentration of the poly(rA)$_{1100}$ in the reaction mixture. But, only those binding sites which also had an oligo(dT) primer associated with them could be productive in terms of poly(dT) synthesis, making the other poly(rA) enzyme binding sites competitive inhibitors. Secondly, the formation of triple-stranded helices by poly(rA)·poly(dT) (Riley et al., 1966) could have an inhibitory effect. Thirdly, the polymerase has a strong tendency to remain associated with the template·daughter strand hybrid even after the transcript has been completed, as will be seen in Figure 2. These complex interactions are possible causes for the conflicting kinetic results previously reported (Leis, 1976; Dube & Loeb, 1976).

The results of a template challenge experiment presented in Figure 2 demonstrate that at 20 °C in low ionic strength buffer, the enzyme tends to remain associated with the completed complex of daughter strand and template, releasing from it only slowly as seen by the slow rise in the plateau region. The observed plateau is not due to inactivity of the polymerase since a control reaction in which only oligo(dT) was omitted for 360 min immediately initiated synthesis upon addition of the primer. Neither the dTTP nor the template·primer was limiting since the addition of these fresh reagents at 360 min had virtually no effect, while upon addition of a fresh aliquot of enzyme, synthesis restarted and began to plateau at approximately twice the control level found in the reaction which was not supplemented.

The quantity of dTMP incorporated at the point where synthesis plateaus (270 min) is ~500 nucleotides/enzyme, which is in good agreement with the incorporation expected if each enzyme elongated the daughter strand to the end of the template and remained associated with the complex assuming that initiation sites, on the average, would be at the middle of the poly(rA) template. Total dTMP incorporated after 600 min was also directly related to enzyme concentration but not template·primer concentration, further demonstrating

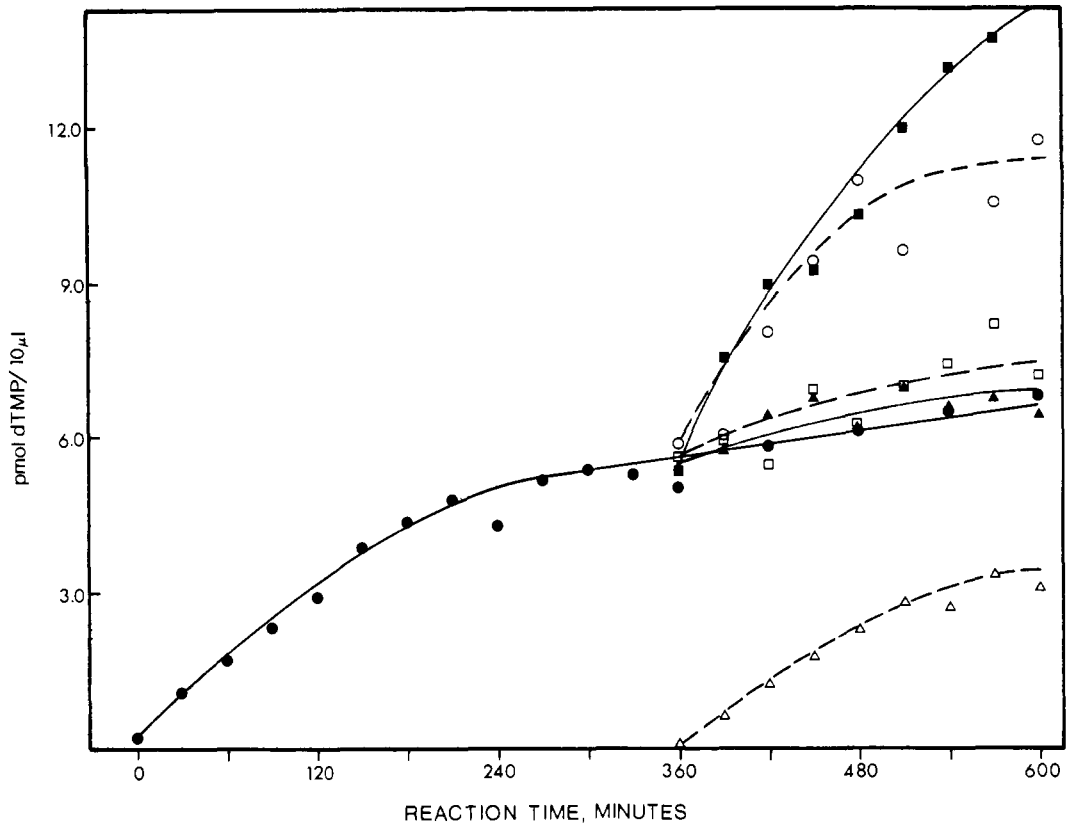

FIGURE 2: Template·primer and enzyme challenge experiment. Reaction mixtures were incubated at 20 °C and contained (●—●) 20 μM [^{3}H]dTTP (specific activity 4.1 Ci/mmol), 50 nM poly(rA)$_{1100}$, 40 nM oligo(dT)$_{12-18}$, 0.2 mM $MnCl_2$, 1 nM AMV polymerase, 5 mM DTT, and 10 mM Tris-HCl, pH 8.3. At 360 min, additional equal quantities of polymerase (○---○), dTTP (▲—▲), poly(rA) and oligo(dT) (□---□), or polymerase, dTTP, and poly(rA)·oligo(dT) (■—■) were added to the reaction. In a separate reaction incubated at 20 °C, oligo(dT) was omitted until 360 min, when it was added to a concentration of 40 nM (△---△).

the slow dissociation of enzyme from the completed hybrids. Increasing the temperature or ionic strength increased the slope of the plateau region, illustrating that the enzyme dissociates more rapidly at higher temperature and ionic strength (data not shown). These results are consistent with the AMV polymerase forming stable, active complexes with the template·primer and agree, in most instances, with those of Uyemura et al. (1975) and Das & Fujimura (1977a,b; 1979), although their analyses concern bacterial polymerases.

Product Predictions Based on the Processive vs. Distributive Mechanisms. Given concentrations of reactants such as 2 μM dTTP, 40 nM poly(rA)$_{1100}$, 20 nM oligo(dT)$_{12-18}$, and 2 nM polymerase, the following poly(dT) product lengths would be predicted based upon the two different mechanisms.

(*1*) *Distributive Hypothesis.* From the above concentrations, it can be seen that there are 100 molecules of dTTP per oligo(dT) and that the ratio of poly(rA) to oligo(dT) primer is 2:1. If every available dTTP was incorporated and the enzymes were randomly distributed among the template·primer complexes by diffusion after each nucleotide addition, the average length of the poly(dT) would be 100 nucleotides plus the length of the oligo(dT)$_{12-18}$ primer to give a total average length of 115 nucleotides. If not all of the dTTP was incorporated, the average length would be correspondingly shorter. In many experiments, 10–15% of the total dTTP was used, or 12 dTTPs added per oligo(dT), giving an expected average length of 27 nucleotides assuming a distributive mode. If some of the enzyme molecules were not active, less product of a smaller size would be made in the given incubation time.

(*2*) *Processive Hypothesis.* From the given concentrations there are 1000 dTTPs/enzyme. If an enzyme initiates polymerization on a template·primer complex where the oligo(dT) hybridizes randomly to the poly(rA) and polymerization continues on that complex until the 5′ end of the poly(rA) is reached, the average poly(dT) product will be 565 nucleotides long. However, poly(dT) from 16 to 1100 nucleotides in length could be made depending on where the oligo(dT) hybridizes on the poly(rA) chain. If not all of the dTTP is used, less product will be made, but the average length would not be shorter unless the polymerization rate was too slow to permit completion of a daughter strand in the allotted time. If the enzyme is not perfectly processive and dissociates before incorporating an average of 550 nucleotides, the mean length will be shorter. The size of the transcripts would not be affected if some of the enzyme molecules were inactive, although less total product would be made.

Even if the polymerase is only moderately processive, there should be a dramatic and easily detectable difference in the products as predicted above. It is a requirement of the arguments presented here that an excess concentration of polymerization initiation sites [poly(rA)·oligo(dT) complexes] over enzyme concentration be used; otherwise, the enzyme would be unable to "distribute" if that was its mechanism.

Size Analysis of the Poly(dT) Transcripts. The elution profiles shown in Figure 3A demonstrate that full-length products similar to those predicted by the processive hypothesis can be synthesized under appropriate conditions. The elution profile expected if all the reaction product in Figure 3A was homogeneous and 565 nucleotides in length is shown by the dashed line. The presence of a significant portion of poly(dT) transcripts less than 565 nucelotides long can be explained by the random hybridization of the oligo(dT) primer to the poly(rA)$_{1100}$ which produces a heterogeneous product. Also, the relatively slow initiation and elongation rates observed at

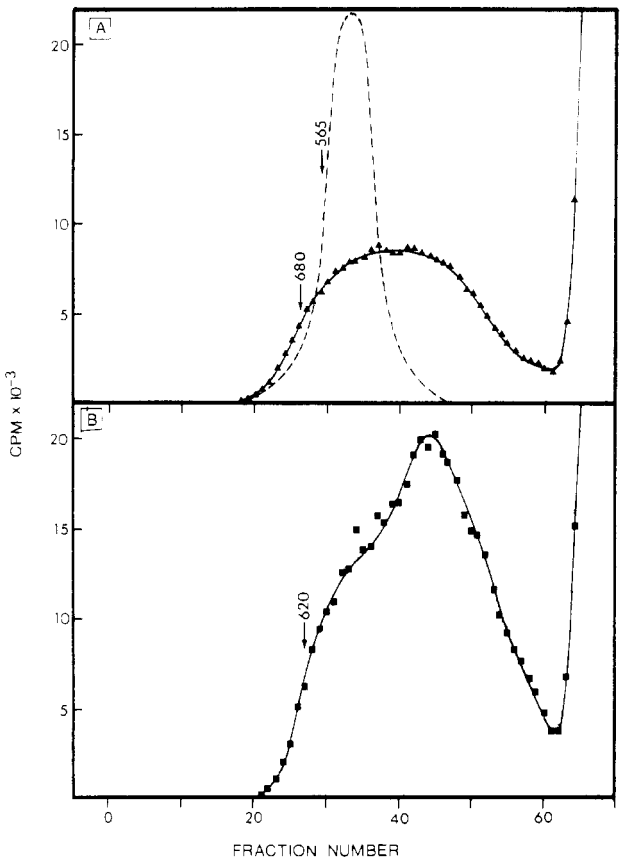

FIGURE 3: Elution profile of poly(dT) products from reactions incubated for 120 min at 25 °C in the presence of 0.2 mM Mn^{2+}. (A) Reactions contained 20 nM $poly(rA)_{1100}$, 20 nM $oligo(dT)_{12-18}$, 3.0 μM dTTP, and 2 nM AMV polymerase (▲). (B) Reactions contained 20 nM $poly(rA)_{1100}$, 40 nM $oligo(dT)_{12-18}$, 6.0 μM dTTP, and 4 nM AMV polymerase (■). All reagents were made in 50 mM Tris-HCl, pH 8.3, 60 mM NaCl, 5mM DTT, and 0.25% NP-40. The observed results are compared with those predicted by the distributive and processive hypotheses in Table I. Since the product is obviously heterogeneous, the smaller products tend to skew the peak toward the smaller sizes. Thus, the value taken from the half-height of the leading edge should be taken as an indication of a minimum for the largest sized pieces synthesized by the polymerase. The dashed line in (A) represents the expected elution profile for a homogeneous, 565 nucleotide long poly(dT) product.

Table I: Analysis of the Products for the Profiles in Figures 3[a] and 5[b]

	Figure						
	3A,	3B,	5A		5B		
	▲	■	●	○	△	■	□
% of total dTTP incorpd	28	34	12	15	5	9	13
av expected length including oligo(dT) if distributive (nucleotides)	57	65	27	30	20	24	28
expected av length if completely processive (nucleotides)	565	565	565	565	565	565	565
obsd av length (nucleotides)	680	325 (620)[c]	350	350	130	250 (620)[c]	250 (620)[c]

[a] Reaction conditions are listed in the legend of Figure 3. [b] Reaction conditions are listed in the legend of Figure 5. [c] The estimated length of pieces in the shoulder of the biphasic peak is 620 nucleotides.

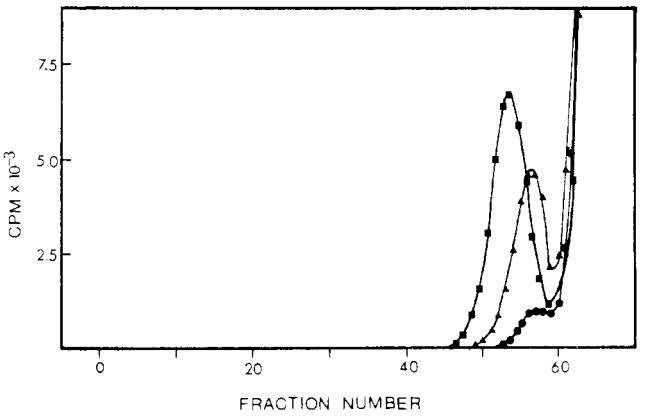

FIGURE 4: Effect of reaction time on the size of the poly(dT) transcripts. Aliquots were removed from a single reaction mixture at 2 (●), 10 (▲), and 60 min (■) containing 20 nM $poly(rA)_{1100}$, 10 nM $oligo(dT)_{12-18}$, 2 nM AMV polymerase, 0.5 μM dTTP, 50 mM Tris-HCl, pH 8.3, 60 mM NaCl, 5 mM DTT, and 0.1 mM $MnCl_2$ incubated at 30 °C.

this lower temperature and dTTP concentration reduce the probability that complete daughter strands will be synthesized before the reaction is quenched.

The expected average product length for the reaction in Figure 3A, assuming a distributive mechanism, is only 57 bases whereas a maximum length of ~680 bases was observed. Product with an average length of 57 nucleotides would elute as a shoulder on the dTTP peak. Since the average expected length assuming perfect processivity is 565 bases, the results clearly demonstrate a processive mechanism. The quantity of dTTP incorporated in these reactions is in good agreement with the results predicted by a scheme in which the enzyme is processive and tends to remain associated with the complex even after reaching the end of the template (Table I). If each enzyme uses an average of 550 nucleotides to reach the end of the template and remains there, 37% of the total dTTP would be incorporated (assuming every enzyme molecule was active), compared with the observed incorporation of 28%.

Increasing the oligo(dT)/poly(rA) ratio to 2:1 skewed the peak toward smaller sizes of product as predicted in the processive model (Figure 3B). The presence of an average of 2 oligo(dT) primers/template should yield transcripts with an average size of 367 nucleotides, which is in good agreement with the observed length of 325 nucleotides. The presence of the 620-nucleotide shoulder can be attributed to that portion of template which had only one primer.

The effect of reaction time on the size and quantity of the poly(dT) has been investigated (Figure 4) by using conditions (30 °C and 110 mM salt) which increase the dissociation of the enzyme from the template·product hybrids, preventing the enzyme from synthesizing full-length transcripts. Also, 0.5 μM dTTP was used, which is well below the estimated K_m of 5 μM of the AMV polymerase (Darling & Reid, 1979), thus reducing the elongation rate. Even though these conditions favor a nonprocessive mechanism, the 118 nucleotide long product made at 2 min of incubation (less than 0.5% of total dTTP incorporated) is longer than could be made if all the available dTTP was incorporated in a distributive manner, is only 20% shorter than the product of a 60-min incubation, and indicates that the rate of addition is at least 59 nucleotides/min. This number clearly represents a lower limit for the elongation rate. Also, since product made in 2 min is almost as long as the product made in 60 min, the data strongly suggest that the rate-limiting step is the initiation of polymerization. If the rate-limiting step was not initiation, then full-length transcripts should be completed in an average of

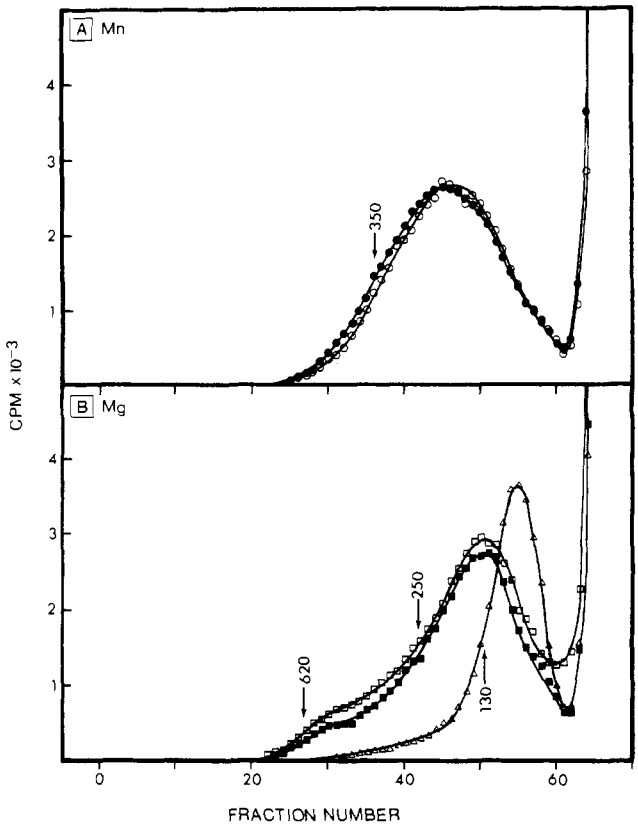

FIGURE 5: Effect of Mg^{2+} and Mn^{2+} metal ions on reverse transcription. All of the above reactions contained 50 nM poly(rA)$_{1100}$, 20 nM oligo(dT)$_{12-18}$, 2 μM dTTP, and 2 nM AMV polymerase and were incubated at 38 °C for 60 min. Additional reagents are listed below. (See Table I for the data pertinent to the predictions based on the distributive and processive hypotheses.) (A) Reactions in 0.2 mM Mn^{2+} with (○) and without (●) 24 μM dATP, dCTP, and dGTP. (B) Synthesis in 6.0 mM Mg^{2+} with (□) and without (■) 24 μM dATP, dCTP, and dGTP and reaction in 0.6 mM Mg^{2+} (△). All reagents were made in 50 mM Tris-HCl, pH 8.3, 60 mM NaCl, 5 mM DTT, and 0.25% NP-40.

9 min assuming the suboptimal elongation rate of 59 nucleotides/min.

The complex interactions of the metal ions Mg^{2+} and Mn^{2+} with deoxyribonucleoside triphosphates and template·primer polymers have been attributed to the formation of coordination complexes between the metal ions and phosphates. These effects are particularly noticeable and troublesome in the endogenous reactions of retroviruses (Vamakopoulas et al., 1977; Rothenberg & Baltimore, 1976, 1977; Clayman et al., 1979). It is clear from the results of Figure 5 and Table I that the metal ions and their concentrations do affect the size and quantity of the poly(dT) transcripts, but in every case, the polymerase is processive. The product length predicted by the distributive hypothesis, based on 5–15% dTTP incorporation, should be 30 nucleotides or less, which is much smaller than the observed products. A 10-fold decrease in Mg^{2+} concentration led to a substantial reduction in product length but yielded surprisingly homogeneous transcripts. The addition of dATP, dCTP, and dGTP at 24 μM had little or no effect on the reactions.

Discussion

Our data indicate that the enzyme·template·primer complex is more stable at low temperature and ionic strength and that longer products are made under these conditions than at high temperature and ionic strength where the complex is less stable. Other factors which alter either the polymerization rate or the stability of the complex will influence the degree of processivity.

We have found that incubations of 2, 10, and 60 min yield products of similar length and interpret this to mean that, at least in vitro, initiation is the rate-limiting step under these conditions. Our estimates of the in vitro elongation rate suggest a minimum of 60 nucleotides/min. This estimate is a minimum since the sensitivity of product detection and the suboptimal reaction conditions used do not allow accurate determinations of product size at shorter reaction times where few enzyme molecules have initiated.

An alternative explanation for our conclusion of processivity is that slippage of either the poly(rA) or the poly(dT) could allow the enzyme to sit in one place throughout the polymerization. This mechanism is not consistent with our results since no plateau region would be observed as found in Figure 2. It could also be argued that once a daughter strand has become elongated, it becomes a better "primer" than the oligo(dT) and is more likely to be further elongated, thus giving apparent processivity by a distributive mechanism. This is unlikely as the only way one daughter strand could become significantly longer than the others is if the enzyme were processive since a distributive polymerase would elongate the daughter strands evenly and no advantage would be given to any of the growing poly(dT) strands. RNase H activity associated with the polymerase could not increase the size of the transcripts and does not detract from our conclusions.

The mechanisms by which metal ions participate in and influence transcription are unknown, but it is clear from our data that reverse transcription is processive with either Mn^{2+} or Mg^{2+} and that the length and quantity of the products are dependent on the type and concentration of the metal ions. Metal ions such as Mg^{2+} and Mn^{2+} are known to participate in the catalysis of polymerization and to influence the secondary structure of the template (Vamvakopoulos et al., 1977). The homogeneity of the product made in the presence of 0.6 mM Mg^{2+} (Figure 5B) suggests that the secondary structure of the template·primer complex can determine the length of the transcripts.

Similar experiments done with the reverse trancriptase from a hamster RNA tumor virus demonstrate that it also has a processive mode of action (unpublished experiments). We have previously shown the hamster viral polymerase to be closely related biochemically and immunologically to the murine leukemia virus polymerase (Gregerson et al., 1979). The only other experiments done with mammalian DNA polymerases, the α and β DNA polymerases from calf thymus, suggested that they were "quasi-processive" since they incorporated an average of only 8–11 nucleotides before enzyme dissociation (Das & Fujimura, 1979).

The procedures described here are conceptually simple and should be readily applicable to other systems. Previous attempts to determine the mechanism of reverse transcription relied on kinetic analyses of data which were complicated by the apparent nonclassical kinetics of the polymerase reactions (Leis, 1976; Dube & Loeb, 1976). Kinetic arguments can be used to rule out many possibilities but can never prove a mechanism. The complications include the rate-limiting nature of initiation, the slow dissociation of polymerase from the complexes, substrate inhibition by excess template, and conformational effects such as the formation of triple-stranded helices (Riley et al., 1966). A number of recent publications have reported both processive and distributive mechanisms for *E. coli* DNA Pol I by using primarily kinetic data. The only other DNA polymerase shown to be processive is the T5

bacteriophage induced DNA polymerase which was studied by using both kinetic and product size analyses (Das & Fujimura, 1977a,b).

Acknowledgments

We thank Frederic Richards for helpful suggestions during the preparation of the manuscript, William Cosina for help with preliminary studies, Sherman Weissman for SV-40 DNA fragments, and William Melchior for many thought provoking discussions.

References

Bambara, R. A., Uyemura, D., & Choi, T. (1978) *J. Biol. Chem. 253*, 413-423.
Chang, L. M. S. (1975) *J. Mol. Biol. 93*, 219-235.
Clayman, C. H., Mosharrata, E., & Faras, A. J. (1979) *J. Virol. 29*, 242-249.
Darling, T. L., & Reid, T. W. (1979) *Nucleic Acids Res. 6*, 1189-1201.
Darling, T., Albert, J., Russell, P., Albert, D. M., & Reid, T. W. (1977) *J. Chromatogr. 131*, 383-390.
Das, S. K., & Fujimura, R. K. (1977a) *J. Biol. Chem. 252*, 8700-8707.
Das, S. K., & Fujimura, R. K. (1977b) *J. Biol. Chem. 252*, 8708-8712.
Das, S. K., & Fujimura, R. K. (1979) *J. Biol. Chem. 254*, 1227-1239.
Dube, D. K., & Loeb, L. A. (1976) *Biochemistry 15*, 3605-3611.
Gregerson, D. S., Russell, R., & Reid, T. W. (1979) *J. Gen. Virol. 43*, 327-337.
Leis, J. (1976) *J. Virol. 19*, 932-939.
Maniatis, T., Jeffrey, A. Y., & Kleid, D. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1184-1188.
McClure, W. R., & Jovin, T. M. (1975) *J. Biol. Chem. 250*, 4073-4080.
Riley, M., Maling, B., & Chamberlin, M. J. (1966) *J. Mol. Biol. 20*, 359-389.
Rothenberg, E., & Baltimore, D. (1976) *J. Virol. 17*, 168-174.
Rothenberg, E., & Baltimore, D. (1977) *J. Virol. 21*, 168-178.
Sherman, L. A., & Gefter, M. L. (1976) *J. Mol. Biol. 103*, 61-76.
Uyemura, D., Bambara, R., & Lehman, I. R. (1975) *J. Biol. Chem. 250*, 8577-8584.
Vamvakopoulos, N. C., Vournakis, J. N., & Marcus, S. L. (1977) *Nucleic Acids Res. 4*, 3589-3597.

Primary Structure of Murine Major Histocompatibility Complex Alloantigens: Amino Acid Sequence of the Amino-Terminal One Hundred and Seventy-three Residues of the H-2K^b Glycoprotein†

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ABSTRACT: The amino-terminal 173 residues of the murine histocompatibility antigen H-2K^b have been assigned by using radiochemical methodology. The complete sequence of an 86 residue glycopeptide (CN-Ib), which is one of the five major CNBr fragments of K^b, was determined by analysis of peptides obtained from digests using thrombin and V8 staphylococcal protease. Complete sequences were obtained for the three large thrombic peptides, and these were aligned by using peptides from the V8 protease digest. Alignment of the CNBr fragments was carried out by using [^{35}S]Met-labeled peptides from a tryptic digest of the papain-cleaved H-2K^b molecule. Positive identification was possible for all the common amino acids except Asp (*Asp*) which was indirectly assigned and which is designated in italics. The sequence obtained in our studies was Gly-Pro-His-Ser-Leu-Arg-Tyr-Phe-Val-Thr-Ala-Val-Ser-Arg-Pro-Gly-Leu-Gly-Glu-Pro-Arg-Tyr-Met-Glu-Val-Gly-Tyr-Val-*Asp*-*Asp*-Thr-Glu-Phe-Val-Arg-Phe-*Asp*-Ser-*Asp*-Ala-Glu-Asn-Pro-Arg-Tyr-Glu-Pro-Arg-Ala-Arg-Trp-Met-Glu-Gln-Glu-Gly-Pro-Glu-Tyr-Trp-Glu-Arg-Glu-Thr-Gln-Lys-Ala-Lys-Gly-Asn-Glu-Gln-Ser-Phe-Arg-Val-*Asp*-Leu-Arg-Thr-Leu-Leu-Gly-Tyr-Tyr-(Asn)-Gln-Ser-Lys-Gly-Gly-Ser-His-Thr-Ile-Gln-Val-Ile-Ser-Gly-Cys-Glu-Val-Gly-Ser-*Asp*-Gly-Arg-Leu-Leu-Arg-Gly-Tyr-Gln-Gln-Tyr-Ala-Tyr-*Asp*-Gly-Cys-*Asp*-Tyr-Ile-Ala-Leu-Asn-Glu-*Asp*-Leu-Lys-Thr-Trp-Thr-Ala-Ala-*Asp*-Met-Ala-Ala-Leu-Ile-Thr-Lys-His-Lys-Trp-Glu-Gln-Ala-Gly-Glu-Ala-Glu-Arg-Leu-Arg-Ala-Tyr-Leu-Glu-Gly-Thr-Cys-Val-Glu-Trp-Leu-Arg-Arg-Tyr-Leu-Lys. These data represent the longest reported amino acid sequence determined by utilizing radiochemical methodology and provide the first extensive information on the primary structure of murine histocompatibility antigens.

The major histocompatibility complex (MHC)[1] [see reviews by Klein (1975), Shreffler & David (1975), Vitetta & Capra (1978), Snell et al. (1976), Goetze (1977), and Festenstein & Demant (1979)] is a linked set of genes, the products of which have important roles in host reaction to antigenic stimulation. The murine MHC, which encompasses the original H-2 transplantation locus defined by Gorer (1936), is presently defined as the genetic material on chromosome 17 from the K region (nearest to the centromere) to the TLa region, a distance of about 1.5 cmorgans away.

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[1] Abbreviations used: MHC, major histocompatibility complex; PTH, phenylthiohydantoin.

0006-2960/80/0419-0306$01.00/0