KOREAN JOURNAL OF ZOOLOGY
Vol. 22, No. 4, 141—152 (1979)

Biochemical and Immunological Characterization of the DNA Polymerase and RNase H in Feline Leukemia Virus

Hyune Mo Rho
(Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland)

고양이 백혈병 바이러스의 DNA Porymerase와 RNase H의 생화학적 및 면역학적 연구

노 현 모
(암세포 생물학 연구실, 미 국립 암연구소)

(Received September 1, 1979)

적 요

고양이 백혈병 바이러스에서 reverse transcriptase를 분리하여 생화학적 및 면역학적 연구를 하였다. 분자량은 72,000이고, DNA polymerase와 RNase H의 활성은 0.05—1 mM M_n^{2+}와 50—80 mM KCl에서 가장 좋았다.

DNA polymerase와 RNase H는 같은 단백질 분자에 있으며, chymotrypsin 처리로서 RNase H를 쪼개낼 수 있으며, 이 RNase H도 reverse transcriptase의 항체에 의해서 활성이 거의 억제 된다. Reverse transcriptase의 항체 결합위치와 활성을 내는 위치는 다른 것 같다.

INTRODUCTION

The biological function of reverse transcriptase in RNA tumor viruses is not yet fully understood, but the enzyme might play a key role in the integration of viral genome (or part of it) into host DNA by the production of a DNA copy of the viral RNA. Most purified DNA polymerases from avian, rodent, canine, and simian RNA tumor viruses exhibit two enzyme activities; RNA dependent DNA polymerase and RNase H. The function of RNase H during the synthesis of proviral DNA remains obscure. However, it has been proposed that during the synthesis of complementary DNA, RNase H degrades the RNA template and provides a primer with a 3′-OH to initiate the synthesis of the second strand of DNA

(Verma, 1977; Sarngadharan *et al.*, 1978).

Recently, we have become interested in the detailed biochemical and immunological properties of DNA polymerase and RNase H in the reverse transcriptase from feline leukemia virus; first, because antibodies to DNA polymerase can be a sensitive indicator of natural infection of cats by feline leukemia virus (Jacquemin *et al.*, 1978; Rho and Gallo, 1979); second, because immunoglobin molecules which react with DNA polymerase from feline leukemia virus have been found on the surface of some human leukemia blood cells (Jacquemin *et al.*, 1978); third, because sensitive approaches toward the detection of either viral DNA polymerase or RNase H activities have becoming increasingly important in studies of viral carcinogenesis. In this report, feline viral reverse transcriptase has been purified and characterized by biochemical and immunological techniques. Proteolytic digestion of the reverse transcriptase was able to generate RNase H activity which was neutralized by antibody to the reverse transcriptase.

MATERIALS AND METHODS

Materials.

Theilen strain of feline leukemia virus (FeLV) was grown in a suspension culture of a cat embryo cell line (FL-74), and purified by sucrose density gradient. Antisera against purified reverse transcriptase of FeLV and simian associated virus (SSAV) were prepared in goats as described (Rho and Gallo, 1979). Other antisera were purchased from Cappel Lab. [^{3}H]-labeled nucleotides, and homopolymers were obtained from New England Nuclear Co., and oligomer-homopolymer and homopolymer-agarose from P.L. Biochemicals. *E. coli* DNA I polymerase was generously supplied by Dr. Loeb, Fox Chase Cancer Institute, Philadelphia

Purification of FeLV reverse transcriptase.

FeLV (50 mg) was pelleted at 100,000 xg for 90 min at 4° C. The resulting pellet was resuspended in 25 ml of Buffer A containing 50 mM Tris-HCl (pH 7.9), 500 mM KCl, 5 mM dithiothreitol (DTT), 20% glycerol, 0.5% Triton X-100, and 1 mM phenylmethyl-sulfonyl fluoride (PMSF).

The suspension was sonicated by six 30 second bursts at maximum output with a microtip using a Branson model sonifier and cleared by centrifugation at 30,000 xg for 1 hr. After adjustment of KCl concentration to 0.4 M in the same buffer, the supernatant was chromatographed on a DEAE-cellulose column to remove nucleic acids. The flow-through was dialyzed overnight against Buffer A containing 20 mM N, N-bis [2-hydroxyethyl]-2-aminoethane sulfonic acid (BES) (pH 6.5), 1 mM EDTA, 0.1% Triton X-100, 100 mM KCl, 20% glycerol, and 1 mM PMSF. The dialyzed preparation was applied to a phosphocellulose (pll) col-

umn (1.5×10 cm) previously equilibrated with Buffer B. After it had been washed with more than 50 ml of the same buffer, the polymerase was eluted with an 100 ml of linear 0.1 to 1.0 M KCl gradient in the buffer. Figure 1A shows the elution profile of enzyme activity from pll column. The reverse transcriptase was eluted between 0.35 and 0.45 M KCl. The enzyme peak was pooled and dialyzed against Buffer C containing 50 mM Tris-HCl (pH 7.5), 100 mM KCl, 1 mM DTT, 5 mM $MgCl_2$, 0.1% Triton X-100, 20% glycerol, and 1 mM PMSF, and further purified by sequential nucleic acid affinity chromatographies as described (Grandgenett and Rho, 1975). The dialyzed enzyme was loaded to a poly (C)-Sepharose column (1.0×5 cm). The column was washed with 30 ml of Buffer C and eluted with a total 80 ml of a linear gradient of 0.1 M to 1.0 M KCl in the buffer. The enzyme was eluted between 0.2 to 0.30 M KCl (Fig. 1B). The peak fractions were pooled and diluted 5 fold with Buffer D containing 50 mM Tris-HCl (pH 7.4), 1 mM DTT, 0.01% Triton X-100, and 20% glycerol. The diluted enzyme was loaded on the poly(A)-oligo(dT)-cellulose colum (1.5×10 cm) and washed with 50 ml of Buffer D containing 0.1 M KCl. The enzyme was eluted with a 100 ml of a linear gradient of 0.1 M to 1.0 M KCl in the buffer. The enzyme peak, eluted between 0.25 M and 0.32 M KCl (Fig. 1C), was concentrated by dialyzation against Buffer E containing 100 mM Tris-HCl (pH 7.9), 100 mM KCl, 0.001% Triton X-100, 2 mM DTT, and 20% Ficoll. The concentrated enzyme was added to the equal volume of cold glycerol and stored at -20° C for a further characterization.

DNA polymerase assays.

The standard reaction mixture for DNA polymerase activity in 0.1 ml contained 50 mM Tris-HCl (pH 7.9), 1 mM DTT, 0.05 mM $MnCl_2$, 50 mM KCl, 0.01% Triton X-100, 20% glycerol, 10 μg BSA, 1 μg of poly (rA)·oligo (dT), 15 μM of [^{3}H]TTP (50 Ci/m mol). When AMV 70S RNA was used as template, 0.1 mM $MnCl_2$, 15 μM of [^{3}H]TTP, and 100 μM each of dATP, dCTP, dGTP were instead adopted in the standard reaction mixture. When poly (Cm)·oligo (dG) was used as template, 1 mM $MnCl_2$, and 15 μM of [^{3}H]dGTP (30 Ci/m mol) were adopted in the standard reaction mixture. The reaction mixture was incubated at 37° C for 30 min. and synthesized polymer was measured on Whatman DEAE-cellulose (DE-81) paper disks as described (Grandgenett and Rho, 1975).

Synthesis of fd-DNA·[^{3}H] RNA hybrid.

The procedure to synthesize the fd DNA-[^{3}H]RNA hybrid was adopted with some modifications by Sarngadharan *et al.* (1975). Briefly, the reaction mixture (0.1 ml) contained 80 mM Tris-HCl (pH 8.0), 50 mM KCl, 10 mM DTT, 10 mM $MnCl_2$, 0.5 mM each of ATP, GTP, and CTP, 0.14 mM [^{3}H] UTP (35 Ci/m mol)

10 μg of fd-DNA (Miles Co.), and 3 μg of *E. coli* RNA polymerase (holoenzyme). After incubating at 37° C for 6 hr, the hybrid was isolated by pronase and chloroform treatment followed by a Sephadex G-50 chromatography. The hybrid, with specific activity of 10,000 cpm/ng, was resistant more than 90% to RNase A digestion in 50 mM Tris-HCl (pH 7.5), 0.3 M NaCl.

RNase H assay.

The standard reaction mixture for RNase H assay contained, in 0.1 ml, 50 mM Tris-HCl (pH 7.9), 1 mM DTT, 0.05 mM $MnCl_2$, 50 mM KCl, 10 μg of the fd DNA-[^{3}H]RNA hybrid. The reaction mixture was incubated at 37° C for 15 min. and acid-soluble count was measured by adding 40 μg of BSA and 4 μl of 100% trichloracetic acid. The mixture was centrifuged and the superment was counted in liquid scintillation fluid.

Glycerol gradient.

FeLV DNA polymerase was layered on a 10 to 30% glycerol gradient containing 50 mM Tris-HCl (pH 7.4), 2 mM DTT, 0.3 M KCl, 0.05 mM $MnCl_2$, 0.1% Triton X-100. The gradient was centrifuged for 18 hr at 45,000 rpm at 4° C in an SV 50.1 rotor of a Spinco ultracentrifuge. Fractions were collected from the bottom of the centrifuge tube, and aliquots from each fraction were assayed for DNA polymerase and RNase H activities. Parallel gradients were run using *E. coli* DNA polymerase I and bovine serum albumin (BSA) as standard markers. *E. coli* DNA polymerase activity was measured with poly (dA)·oligo (dT) as template-primer. The position of BSA in the gradient was identified by a slab polyacrylamide gel electrophoresis followed by Coomasie blue staining.

Polyacrylamide gel electrophoresis.

Polyacrylamide gel electrophoresis in the presence of 0.1% SDS (SDS-PAGE) was performed according to the method of Laemmli (1970). Polyacrylamide gels (10%) were stained with Coomassie blue and destained with 7.5% acetic acid.

Immunologic assays.

The neutralization assay and double antibody immunoprecipitation were performed as previously described (Smith *et al.*, 1975), with a minor modification. Briefly, FeLV reverse transcriptase plus primary antibody (Goat IgG) was incubated for 4 hr at 4° C in a 50 ul reaction mixture containing 50 mM Tris-HCl (pH 7.8), 50 mM KCl, 0.05 mM $MnCl_2$, 0.01% Triton X-100, 1 mg/ml BSA. The excess amount of second antibody (rabbit IgG against goat) was then added to the reaction mixture to precipitate the primary antibody. After further incubation for 17 hr at 4° C the reaction mixture was diluted 4 fold, one series of rea-

ction mixture was centrifuged for 5 min. in a Beckman microfuge B. The enzyme activity remaining in the supernatant of the centrifuged samples and an aliquot of the non-centrifuged samples were determined in the standard reaction mixture. The amount of enzyme was expressed as percent of control activity, based on the amount of polymerase activity remaining when the primary antibody consists solely of nonimmune IgG. The amount of second antibody was properly titrated for a maximal precipitation. All antibodies and BSA before use were treated with 2 mM PMSF and cleared by centrifugation.

Proteolysis of FeLV reverse transcriptase by chymotrypsin.

FeLV reverse transcriptase (about 4 μg), which was purified by additional affinity column poly (G)-Sepharose, was diluted with buffer containing 50 mM Tris-HCl (pH 7.4), 0.05 mM $MnCl_2$, 0.3 M KCl, 5 mM DTT, 0.1% Triton X-100, 20% glycerol. The enzyme was digested with 1 μg chymotrypsin at 37° C until that the remaining polymerase activity is about 40% as described (Lai and Verma, 1978). The reaction was stopped by addition of 2 mM PMSF and adjusted to the final concentration of KCl and DTT to 1.5 M and 10 mM, respectively. The reaction mixture was incubated at 4° C for 2 hr with occassional shaking, and then diluted with a buffer containing 50 mM Tris-HCl (pH 7.4), 0.05 mM $MnCl_2$, 5 mM DTT, 0.1% Triton X-100, and 20% glycerol, to lower KCl concentration to the final 20 mM. The diluted reaction mixture was then loaded on a poly(G)-agarose column (0.5×4 cm) previously equilibrated with the dilution buffer, and washed with 10 ml of dilution buffer. The enzyme was eluted with a linear gradient of 30 ml of 0 to 0.8 M KCl in the buffer. The polymerase and RNase H were assayed through fractions as described in the method.

RESULTS

Purification of FeLV reverse transcriptase.

Feline leukemia virus enzyme extract was passed through DEAE-cellulose column to remove nucleic acids. The enzyme was then chromatographed on a phosphocellulose at pH 6.5 (Fig. 1A). At this pH, reverse transcriptase was eluted with 0.4 M KCl. The major glycoprotein (gp70) was eluted between 0.1 to 0.2 M KCl and major group specific internal protein (p30) between 0.2 to 0.3 M KCl as reported (Strand and August, 1973). The phosphocellulose chromatography at pH 6.5 separated these three viral proteins with a minor cross-contamination. If the phosphocellulose column was run with a buffer at pH 7.5 or 7.8, the separation of these three antigens was poor, and large amount of p30 cochromatographs with reverse transcriptase (SDS-PAGE data not shown). Subsequent chromatographies on poly(C)-Sepharose and the hybrid affinity column, poly(A)·

oligo(dT)-cellulose usually provided a relatively homogeneous reverse transcriptase (Fig. 1 and 3). However, additional affinity chromatography steps with poly(G)-agarose and Lentile-lectin-agarose gave a reverse transcriptase free of a single-stranded specific RNase and glycoproteins (data not shown).

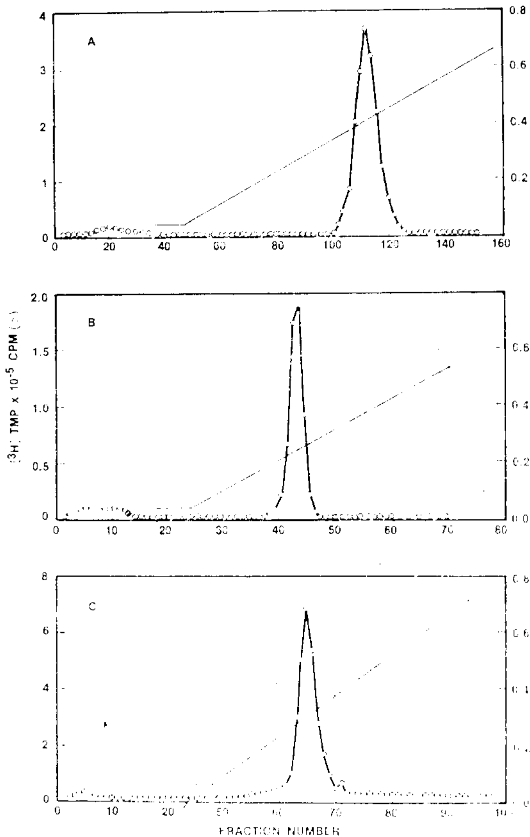

Fig. 1. Purification of FeLV reverse transcriptase by ion exchange and sequential affinity chromatography. Fractions were assayed for RNA-directed DNA polymerase activity with poly(rA)·oligo(dT) as described in the text. Salt concentrations were measured conductimetrically. (A)phosphocellulose chromatography; (B) poly (C)-Sepharose chromatography; (C) poly (A)·oligo (dT)-cellulose chromatography.

Determination of molecular weight.

An aliquot of the purified reverse transcriptase was adjusted to the same buffer strength as in the glycerol gradient and layered on 10 to 30% glycerol gradient for a centrifugation. *E. coli* DNA polymerase I and bovine serum albumin were run on parallel gradients as markers. Fig. 2 shows the sedimentation profile of polymerase activity with poly(rA)·oligo(dT) and poly(Cm)·oligo(dG) as template-primer. RNase H activity appeared at the same position as the polymerase activity on the gradient. Assuming the marker proteins behaved in a similar way in the sedimentation, FeLV reverse transcriptase was found to be about 72,000 daltons.

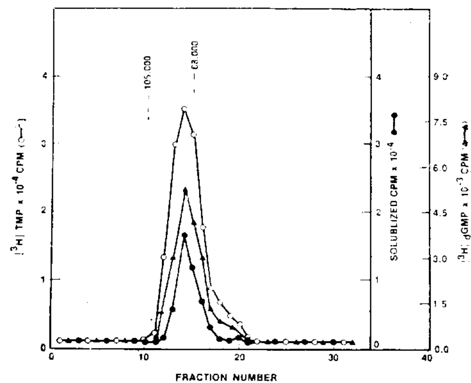

Fig. 2. Glycerol gradient sedimentation of purified FeLV reverse transcriptase. The gradient was centrifuged fractionated as described in the text. Aliquots of each fraction were assayed for DNA polymerase activity with poly (A)·oligo (dT) (○) and with poly (Cm)·oligo (dG) (▲). RNase H activity (●) was analyzed with [^{3}H]-fd hybrid.

This value is very similar to the reported molecular weight estimates by gel filtration in 6 M guanidine-HCl (Tronick *et al.*, 1972). Fig. 3 shows

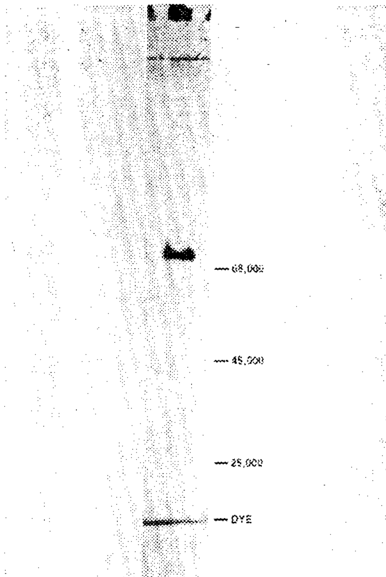

Fig. 3. SDS-PAGE analysis of purified FeLV reverse transcriptase. Reverse transcriptase was subjected to electrophoresis on a 10% slab gel, and the gel was stained with Coomassie blue. The following proteins were used as markers: bovine serum albumin, molecular weight 68,000; ovalbumin, molecular weight 45,000; and chrymotrypsin, molecur weight 25,000.

SDS-PAGE analysis of the purified FeLV reverse transcriptase, revealing a single polypeptides of molecular weight 72,000 relative to the marker protein bovine serum albumin. These results suggest that FeLV reverse transcriptase consists of a single polypeptides of 72,000 daltons.

Biochemical properties of FeLV reverse transcriptase.

The DNA polymerase and RNase H activities were copurified through several steps of purifications by ion-exchange and affinity chromatographies, and glycerol gradient centrifugation. These results suggest that both enzyme activities reside on the same polypeptides molecule as reported in other retroviruses (see recent reviews by Verma, 1977 and Sarngadharan *et al.*, 1978).

The choice of divalent cation with various template-primers can markedly affect the transcription efficiency by FeLV DNA polymerase (Table 1). At the optimum concentration of Mn^{2+} (0.05—0.1 mM), Poly(rA)·oligo(dT)-directed poly (dT) synthesis was four times more efficient than at the optimum Mg^{2+} concentration (5 mM). The cation requirement with modified template-primer (poly(rCm)·oligo(dG)) or natural template (AMV 70S RNA) was higher than poly(rA)·

Table I Efficiency of utilization of various template-primers by FeLV DNA polymerase

Template-Primer*	Optimum divalent cation (mM)	[^{3}H]-Substrate	pmole Incorporated
poly (rA)·oligo $(dT)_{12-18}$	Mg^{2+} (5)	TTP	28.49
	Mn^{2+}(0.05)	TTP	121.00
poly(rCm)·oligo$(dG)_{12-18}$	Mg^{2+}(20)	dGTP	1.00
	Mn^{2+}(1)	dGTP	6.20
poly(dA)·oligo$(dT)_{12-18}$	Mg^{2+}(10)	TTP	<0.70
	Mn^{2+}(10)	TTP	<0.60
AMV 70S RNA	Mg^{2+}(10)	TTP	0.10
	Mn^{2+}(0.1)	TTP	1.24

*The amount of synthetic template-primer was 1μg per assay and AMV 70 S RNA was 0.58 μg per assay.

oligo(dT) (Table 1). In particular, the optimum concentration of Mn^{2+} (Fig. 4A) appeared to be lower with FeLV DNA polymerase than reported value with the enzymes from MuLV (Verma, 1975; Gerad and Grandgenett, 1975). This optimum concentration was not changed even though the concentration of salts (KCl and NaCl) being changed (Fig. 4B). The optimum concentration of salts appeared to be between 50 mM to 80 mM (Fig. 4B), which is similar to the reported value (Verma, 1977; Sarngadharan *et al.*, 1978). The optimum concentration of cation and salts for the RNase H activity of FeLV reverse transcriptase was similar to those of DNA polymerase (Fig.5A and B). However, the RNase H could use Mg^{2+} to catalize the degradation of fd DNA-[^{3}H]RNA hybrid (Fig. 5A). These results are similar to the report observed for MuLV reverse transcriptase (Verma, 1975).

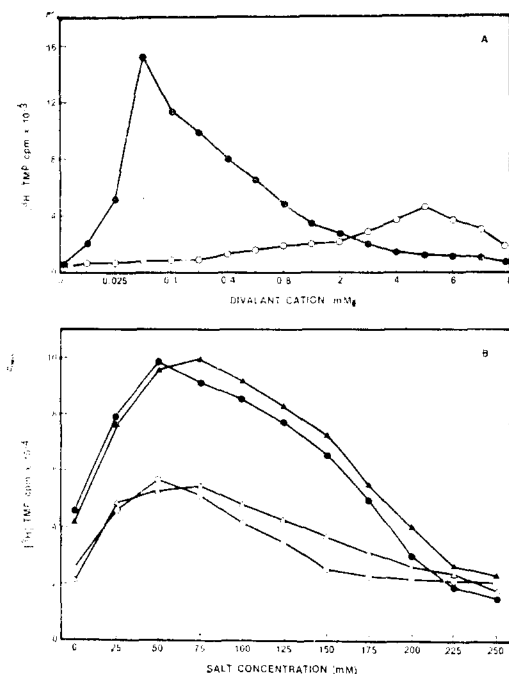

Fig. 4. Effects of cation and salt concentration on FeLV reverse transcriptase with poly (A)·oligo (dT). (A) Divalent cation preference of the enzyme in the presence 50 mM KCl were assayed with different concentration of Mn^{2+} (●) and Mg^{2+} (○). (B) Effect of KCl (●) and NaCl (▲) on the enzyme activity were assayed in the presence of 0.05 MM Mn^{2+}. Effect of KCl (○) and NaCl (△) was also checked in the presence of 0.5 mM Mn^{2+}, which has been known as optimum concentration.

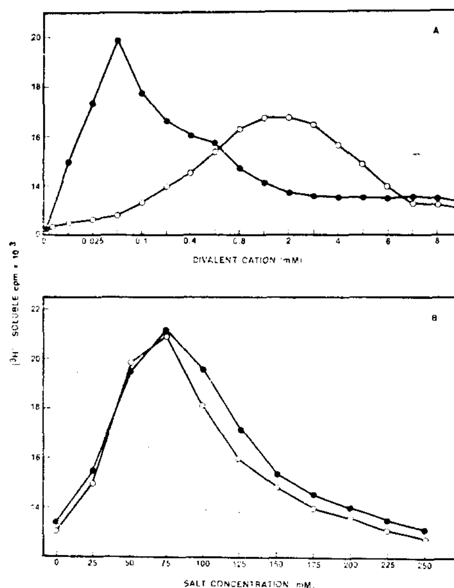

Fig. 5. Effects of cation and salt concentration on RNase H activity with [^{3}H]-fd-Hybrid. (A) Divalent cation preference of the RNase H activity in the presence of 50 mM KCl were assayed with different concentration of Mn^{2+} (●) and Mg^{2+} (○). (B) Effect of KCl (●) and NaCl (○) were assayed in the presence of 0.05 mM Mn^{2+}.

Neutralization and antibody binding assay.

Antibodies prepared against DNA polymerase or disrupted virions have been shown to possess neutralization activities (Scolnick *et al.*, 1974; Todaro and Gallo, 1973) and binding properties (Panet *et al.*, 1975; Krakower *et al.*, 1977; Rho and Gallo, 1979). The FeLV DNA polymerase was tested for the relationship of the binding of immunized IgG to the enzyme and its resulting neutralization activity as described in the method. Fig. 6 shows that the residual polymerase activity was decreased up to 10—30% when the immune complex was removed by centrifugation, whereas the controls did not. Similar observations were reported with DNA polymerase γ (Robert-Guroff and Gallo, 1974). These results suggest that the binding of antibody to reverse transcriptase does not completely inhibit the DNA polymerase activity. Alternatively, this results may be interpreted that the immunogenic and catalytic sites are different.

Generation of RNase H by chymotrypsin.

FeLV reverse transcriptase was isolated by ion-exchange and nucleic acid affinity chromatographies. The purified enzyme consists of a single polypeptides

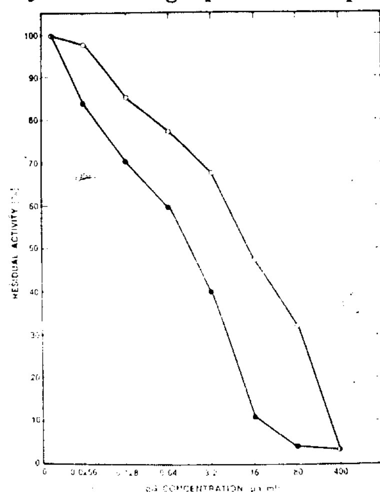

Fig. 6. Enzyme neutralization by double antibody immunoprecipitation assay. The enzymen eutralization assays were carried out in duplicate as described in the test. The residual enzyme activities were measured before separation of the antibody-bound reverse transcriptase (○) and after separation by centrifugation (●).

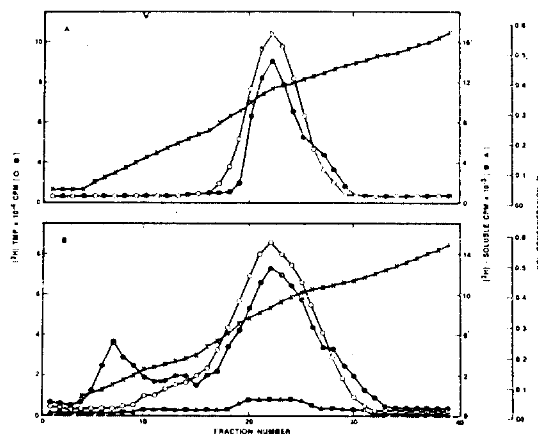

Fig. 7. Poly (G)-Sepharose chromatography of Fe LV reverse transcriptase before and after limited digestion with chymotrypsin. Aliquots from each fraction were assayed for DNA polymerase and RNase H activities as described in the text. (A) control; (B) enzyme activities after digestion with chymotrypsin. DNA polymerase activity was assayed in the absence (○) and presence of antibodies (■); RNase H activity was also assayed in the absence (●) and presence of antibodies (▲).

with an estimated molecular weight of 72,000 and exhibits the properties of a viral reverse transcriptase containing both DNA polymerase and RNase H activities. To see if these two enzyme activities are located on the different position of the polypeptide molecule, the poly(G)-Sepharose purified FeLV reverse transcriptase was mildly digested with chymotrypsin and rechromatographed on poly, (G)-Sepharose column as described in the method. The chymotrypsin digestion caused degradation of the reverse transcriptase activities as well and give rise to a new RNase H peak eluted at 0.1 M KCl in the gradient (Fig. 7B). This peak did not show any significant DNA polymerase activity. However, the RNase H activity was completely inhibited by antisera prepared against FeLV DNA polymerase. This results suggest that the new RNase H peak was originated from the FeLV reverse transcriptase by proteolytic cleavage. Final proof of the parent-product relationship will await biochemical and peptide analysis. Similar observation on the separate location of the two enzyme activities have been reported with murine viral reverse transcriptases (Moelling, 1976) and avian enzymes (Verma, 1975). However, it is not known if DNA polymerase alone could be created by proteolytic digestion.

DISCUSSION

In the present study, FeLV reverse transcriptase has been purified and characterized with biochemical and immunologic techniques. Purification of the enzyme by ion-exchange and sequential affinity chromatography yielded a single polypeptide of 72,000 MW. The enzyme catalyzed DNA synthesis using, as template, 70S viral RNA and synthetic polyribonucleotides including poly(Cm). In addition, the purified enzyme demonstrated RNase H activity. Therefore, the 72,000 MW polypeptide is enzymatically characteristic of the type-C viral reverse transcriptase.

Reverse transcriptase has been a major target of investigation as a maker of type-C Virus expression in tumor cells, mainly because its sensitive detection can be achieved by assays using synthetic templates. This enzymatic assays was known to be about ten fold more sensitive than the competitive radioimmunoassys (Panet *et al.*, 1975; Krakower *et al.*, 1977; Rho and Gallo, 1979). However, since certain cellular DNA polymerase (Robert-Guroff and Gallo, 1974) as well as non-type-C viral polymerases (Schlom *et al.*, 1971) can also utilize these templates, detection of enzyme activity alone is insufficient to invoke the presence of a type-C virus. Therefore, the enzymatic assays in conjunction with proper antibodies should be useful in studying viral enzyme expression in tumor tissues.

Another approach to detect viral reverse transcriptase expression is to measure viral RNase H activity, which appears to be more stable than DNA polymerase

(Lai and Verma, 1978; Rho, unpublished observation). The poly(G)-Sepharose purified FeLV reverse transcriptase was mildly digested with chymotrypsin and rechromatographed on poly (G)-Sepharose column. A new RNase H peak appeared at 0.1 M KCl in the gradient. Moreover, this RNase H activity was completely inhibited by antiserum prepared against FeLV reverse transcriptase. This results suggest that the measurement of RNase H activity with a proper antibody should be useful to detect viral enzyme expression in cancer cells.

SUMMARY

Feline leukemia virus DNA polymerase was purified by ion-exchange and nucleic acid affinity chromatographies. The enzyme consists of a single polypeptide chain of approximately 72,000 molecular weight as determined by both of a glycerol density gradient centrifugation and SDS-polyacrylamide gel electrophoresis. The preferred divalent cation for DNA synthesis is Mn^{2+} on a variety of template- primers, and its optimum concentration appears to be significantly lower than reported results of other mammalian type-C viral enzymes. The divalent cation requirement for maximum activity of RNase H is similar to those of DNA polymerase.

Both DNA polymerase and RNase H activities appear to reside on the same molecule as demonstrated by the copurification of both activities through various purification steps. An additional RNase H without detectible polymerase activity was generated by a limited chymotrypsin digestion. This RNase H activity was inhibited equally effectively as RNase H in the intact reverse transcriptase by antisera prepared against reverse transcriptase of feline leukemia virus. Neutralization and binding test showed that antibody binding to reverse transcriptase molecule did not completely inhibit the polymerase acttivity.

ACKNOWLEDGMENTS

The author wish to thank Dr. Robert C. Gallo for his support and helpful discussions.

REFERENCES

Gerad, G., and D.P. Grandgenett. 1975. Purification and characterization of the DNA polymerase and RNase H activities in Moloney sarcoma-leukemia virus. *J. Virol.* **15:** 785—797

Grandgenett, D.P., and H.M. Rho. 1975. Binding properties of avian myeloblastosis virus DNA polymerases to nucleic acid affinity columns. *J. Virol.* **15:** 526—533

Jacquemin, P.C., C. Saxinger, R.C. Gallo, W.D. Hardy, and M. Essex. 1978. Antibody response in cats to feline leukemia virus reverse transcriptase under natural conditions of exposure to the virus. *Virology* **91:** 472—476

Jacquemin, P.C., C. Saxinger, and R.C. Gallo. 1978. Surface antibodies of human myelogenous leukemia leukocytes reactive with specific type-C viral reverse transcriptases. *Nature* **276**: 230—236

Krakower, J., M. Barbacid, and S. Aaronson. 1977. Radioimmunoassay for mammalian type-C viral reverse transcriptase. *J. Virol.* **22**: 331—339

Laemmli, V.K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T_4. *Nature* **227**: 680--685

Lai, M.H.T., and I.M. Verma. 1978. Reverse transcriptase of RNA tumor viruses. V. *In vitro* proteolysis of reverse transcriptase from avian myeloblastosis virus and isolation of a polypeptide manifesting only RNase H activity. *J. Virol.* **25**: 652—663

Moelling, K. 1976. Further characterization of the Friend murine leukemia virus reverse transcriptase-RNase H complex. *J. Virol.* **18**: 418—425

Panet, A., D. Baltimore, and T. Hanafusa 1975. Quantitation of avian RNA tumor virus reverse transcriptase by radioimmunoassay. *J. Virol.* **16**: 146—152

Rho, H.M., and R.C. Gallo. 1979. Characterization of reverse transcriptase from feline leukemia virus by radioimmunoassay. *Virology* **99**: 192—196

Robert-Guroff, M., and R.C. Gallo. 1974. Serological analysis of cellular and viral DNA polymerases by an antiserum to DNA polymerase γ of human lymphoblasts. *Biochem.* **16**: 2874—2880

Sarngadharan, M.G., J.P. Leis, and R.C. Gallo. 1975. Isolation and characterization of a ribonuclease from human leukemic blood cells specific for ribonucleic acid of ribonucleic acid-deoxyribonucleic acid hybrid molecules. *J. Biol. Chem.* **250**: 365—373

Sarngadharan, M.G., M. Robert-Guroff, and R.C. Gallo. 1978. DNA polymerases of normal and neoplastic mammalian cells. *Biochem. Biophys. Acta.* **516**: 419—487

Schlom, J., D.H. Harter, A. Burney, and S. Spiegelman. 1971. DNA polymerase activities in virions of visna virus; a causative agent of a slow neurological disease. *Proc. Natl. Acad. Sci. U.S.A.* **68**: 182—186

Scolnick, E.M., W.P. Parks, and G.J. Rodaro. 1972. Reverse transcriptases of primate viruses as immunological markers. *Science* **177**: 1119—1121

Smith, R.G., J.W. Abrell, B.J. Lewis, and R.C. Gallo. 1975. Serological analysis of human deoxyribonucleic acid polymerases. *J. Biol. Chem.* **250**: 1702—1709

Strand, M., and J.T. August. 1973. Structural proteins of oncogenic ribonucleic acid viruses. *J. Biol. Chem.* **248**: 5627—5633

Todaro, G.J., and R.C. Gallo. 1973. Immunologic relationship of DNA polymerase from human acute leukemia cells and primate and mouse leukemic virus reverse transcriptase. *Nature* **244**: 206—209

Tronick, S.R., E.M. Scolnick, and W.P. Parks. 1972. Reversible inactivation of the deoxyribonucleic acid polymerase of Rauscher leukemia virus. *J. Virol.* **10**: 885—888

Verma, I.M. 1975. Studies on reverse transcriptase of RNA tumor viruses III. Properties of purified Moloney murine leukemia virus DNA polymerase and associated RNase H. *J. Virol.* **15**: 843—854

Verma, I.M. 1977. The reverse transcriptase. *Biochem Biophys Acta.* **473**: 1—38