JOURNAL OF VIROLOGY, Jan. 1981, p. 274-283
0022-538X/80/010274-10$02.00/0

Vol. 37, No. 1

Endodeoxyribonuclease Activity Associated with Rauscher Murine Leukemia Virus

JOHN J. KOPCHICK,[1] JULIE HARLESS,[2] BARBARA S. GEISSER,[3] RONALD KILLAM,[2] ROGER R. HEWITT,[2,3] AND RALPH B. ARLINGHAUS[4]*

Department of Cell Biology, Roche Institute of Molecular Biology, Nutley, New Jersey 07110,[1] and Department of Environmental Biology[2] and Department of Tumor Virology,[4] The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, and The University of Texas Health Science Center Graduate School of Biomedical Sciences,[3] Houston, Texas 77030

Preparations of purified Rauscher murine leukemia virus were found to contain an endodeoxyribonuclease after disruption of the virus with nonionic detergents. The enzyme makes single-strand breaks in linear or covalently closed circular phage double-stranded DNA molecules. The enzyme was partially purified by ion-exchange chromatography on DEAE- and carboxymethyl-Sepharose columns followed by electrophoresis in DNA-containing polyacrylamide gels. The enzyme was separated from reverse transcriptase ($p80^{pol}$), and the final endonuclease preparation contained no detectable reverse transcriptase activity. The DEAE-Sepharose column-purified endonuclease activity contained a polypeptide of about 40,000 M_r that we term p40. Peptide mapping experiments demonstrated that p40 shares methionine-labeled tryptic peptides with $Pr200^{gag\text{-}pol}$ and $Pr135^{pol}$. Six major methionine-labeled tryptic peptides derived from p40 were found in $Pr200^{gag\text{-}pol}$, but only five of these were detected in $Pr135^{pol}$. The four core proteins (p30, p15, pp12, and p10) and $p80^{pol}$ plus p40 account for most, but not all, of the peptide sequences of $Pr200^{gag\text{-}pol}$. The endonuclease-associated p40 is similar in size and precursor origin to the avian retrovirus-coded endonuclease (p32). In view of these similarities to the avian p32 endonuclease and its association with partially purified Rauscher murine leukemia virus-associated endonuclease preparations, we propose that p40 is the Rauscher murine leukemia virus-coded endonuclease.

Retroviruses are known to package a virus-coded RNA-directed DNA polymerase (reverse transcriptase) and its associated activities (DNA-directed DNA polymerase and RNase H) within the core of the mature virus particle. This enzyme complex is involved in the synthesis of the integrated viral DNA. A viral DNA endonuclease has also been detected within the core of avian retroviruses (2, 3, 15, 23). Its role in viral DNA formation and integration is unknown.

The murine leukemia virus (MuLV) reverse transcriptase is a protein with a molecular weight of about 84,000 ($p80^{pol}$) (16, 26) that is formed by synthesis and processing of a joint product of the *gag* and *pol* genes (8, 11, 12, 18) termed $Pr200^{gag\text{-}pol}$. We have recently proposed that other enzymes in addition to the reverse transcriptase are formed by cleavage of $Pr200^{gag\text{-}pol}$ (12), which is synthesized at about one-fortieth the rate of $Pr65^{gag}$ (18), the core protein precursor (1, 8, 20). A detailed analysis of $Pr200^{gag\text{-}pol}$ by peptide mapping showed that the viral core protein sequences (p30, p15, pp12, and p10) and the reverse transcriptase accounted for only about three-fourths of this polyprotein (12). Assuming the M_r of the proteins to be correct, we estimated total M_r of these additional protein sequences to be about 50,000. These additional sequences were detected in both methionine- and tyrosine-labeled tryptic peptide patterns (11, 12). Part of these unidentified sequences were also found in the intermediate reverse transcriptase precursor $Pr135^{pol}$. We have proposed that the cleavage product containing these extra sequences performs an enzymatic function separate from that of reverse transcriptase (11).

In this report, we describe the isolation and partial purification of a DNA endonuclease from virions of Rauscher MuLV (R-MuLV). An approximately 40,000-M_r polypeptide found in virions of R-MuLV was associated with the partially purified endonuclease activity. Tryptic peptide analyses demonstrated that it shares tryptic methionine-containing peptides with $Pr200^{gag\text{-}pol}$ and $Pr135^{pol}$ but appears to be distinct from $p80^{pol}$.

(This research was part of J.J.K.'s Ph.D. dissertation [1980] and B.S.G.'s M.S. dissertation

[1974] presented to the University of Texas Health Science Center Graduate School of Biomedical Sciences, Houston, Tex.)

MATERIALS AND METHODS

Cells and virus. NIH Swiss mouse embryo cells (JLS-V16) infected with R-MuLV were used in this study (19). The culture medium was a modified Eagle formula containing 10% fetal calf serum (24). Virus was produced in 2-quart (ca. 1.89 liters) roller-bottle cultures and purified as described previously (24). [^{3}H]methionine-labeled virus was prepared as described (18).

Analysis of endodeoxyribonuclease activity by velocity sedimentation of ^{14}C-bacteriophage DNA. Bacteriophage T7 was labeled with [^{14}C]thymine during infection of *Escherichia coli* C *thy-321* (6) and purified by precipitation with polyethylene glycol 6000 and isopycnic centrifugation in CsCl gradients (14). T7 DNA was purified by phenol extraction (25). Assay reactions (200 μl) contained 60 mM Tris-hydrochloride (pH 8.1), 20 mM KCl, 10 mm $MgCl_2$, 0.02% Triton X-100, and from 3,000 to 4,000 cpm of ^{14}C-labeled T7 DNA. The amounts of purified R-MuLV analyzed are indicated as amounts of protein added to the reaction mixture. Reactions were terminated by the addition of EDTA to 50 mM and chilling in an ice bath. Total DNA breakage was detected by sedimentation in alkaline sucrose gradients after adjusting samples to 0.1 NaOH, layering onto 4.5-ml linear gradients (5 to 20%) containing 0.3 M NaOH, 0.7 M NaCl, and 0.01 M EDTA. Gradients were centrifuged at 42,000 rpm at 4°C for 2.5 h in an SW50.1 rotor of a Spinco preparative ultracentrifuge. The radioactivity profiles were determined by collecting 9-drop fractions into 5 ml of liquid scintillation fluid (1/25-part Packard Permafluor, 1/3-part Triton X-100, 2/3-part toluene) and counting in a Packard liquid scintillation spectrometer. In some studies reported but not presented in this paper, double-strand DNA breakage was investigated by sedimentation in neutral sucrose gradients after adjusting samples to 0.5% Sarkosyl and layering onto 4.5-ml linear gradients (5 to 20%) containing 1 M NaCl, 0.01 M EDTA, and 0.01 M Tris-hydrochloride, pH 8.5. Centrifugation, gradient fractionation, and radioactivity determinations were performed as for alkaline gradient analysis.

Analysis of endodeoxyribonuclease activity by PM2 DNA breakage. Conditions for performing analysis of endodeoxyribonuclease (endonuclease) activity in polyacrylamide disc gels containing covalently closed circular PM2 DNA or solution assays containing PM2 DNA were as described (5, 13). In brief, endonuclease activity in PM2 DNA gels was accomplished by electrophoresis of samples through PM2 DNA polyacrylamide gels. Gels were then incubated in endonuclease buffer (0.01 M Tris-hydrochloride [pH 8.1]–10 mM $MgCl_2$), chilled, stained with ethidium bromide, and scanned for fluorescence. Regions within the gel containing endonuclease activity are identified by the increased fluorescence associated with enhanced binding of ethidium bromide to broken DNA molecules.

In test tube reactions, the breakage of PM2 DNA is detected by selective denaturation of all but unbroken molecules at pH 12.1, addition of ethidium bromide, and measurement of fluorescence. At pH 12.1 ethidium bromide binds only to unbroken PM2 DNA molecules which are stable to denaturation. Thus, residual fluorescence decreases as PM2 DNA is broken by endonuclease activity (14).

The number of breaks introduced into PM2 DNA molecules during incubation was determined from the following expression: breaks/molecule = ln(If − Imin/Imax − Imin), where Imax = fluorescence of unincubated reaction mixture, If = fluorescence of incubated reaction mixture, and Imin = fluorescence of reaction mixture without DNA. The nuclease activity exhibited single-hit reaction kinetics which could be reliably measured to about 0.05 fractional survival of PM2 molecules. Thus, the assay measures DNA breakage in the range of 0 to 3 breaks/molecule.

Endonuclease purification. About 30 mg of twice-banded virus containing 5×10^7 cpm of [^{3}H]methionine-labeled virus was disrupted in a solution containing 10 mM Tris-hydrochloride (pH 7.5), 5% Nonidet P-40, and 0.5 M KCl (in some cases the 0.5 M KCl was omitted). The mixture was incubated on ice for 30 min and centrifuged at 10,000 × *g* for 10 min. The supernatant was dialyzed against 10 mM Tris-hydrochloride, pH 7.5. The dialyzed solution was applied to a DEAE-Sepharose column (2 by 15 cm) equilibrated with 10 mM Tris-hydrochloride (pH 7.5), and washed with 30 ml of that buffer. The enzyme activities were eluted with a 300-ml linear gradient of NaCl ranging from 0 to 0.5 M. Three-milliliter fractions were collected. Endonuclease activity was assayed with PM2 DNA as described above. The endonuclease activity fractions were pooled, dialyzed against 10 mM Tris-hydrochloride (pH 7.5), and applied to a column (2 by 15 cm) of carboxymethyl (CM)-Sepharose equilibrated with 0.01 M Tris-hydrochloride (pH 7.5), and eluted with a similar NaCl gradient.

As a final step, the CM-Sepharose fractions containing endonuclease activity were fractionated under nondenaturing conditions on a polyacrylamide gel containing PM2 phage DNA, as described (5, 13).

Reverse transcriptase was assayed with Nonidet P-40, Mn^{2+} ion, and poly(rC):oligo(dG) under the conditions of Kacian et al. (9).

Denaturing gel electrophoresis. Fractions containing endonuclease activity eluting from either DEAE-Sepharose or CM-Sepharose columns were pooled and dialyzed against three changes of solution containing 0.01 mM Tris (pH 7.5), 0.0001% sodium dodecyl sulfate (SDS), and 0.0001% mercaptoethanol. After dialysis, the fractions were lyophilized, suspended in electrophoresis sample buffer (8) minus SDS, and loaded onto an SDS-polyacrylamide gel. Electrophoresis and fluorography were performed as described (12).

Peptide mapping. This procedure has been described previously (10).

RESULTS

R-MuLV-associated endonuclease. In preliminary studies, the endonuclease activity associated with R-MuLV at each stage of purification was investigated by determining the pro-

duction of total breaks and double-strand breaks introduced into ^{14}C-labeled T7 DNA. The sequential stages examined included virus pellets from culture medium centrifuged at 10,000 rpm for 4 h, virus sedimented onto 65% sucrose through 20% sucrose, virus sedimenting to equilibrium density in linear sucrose gradients (15 to 60%), and velocity sedimentation of virus through linear sucrose gradients (5 to 20%). Activity that produced single-strand breaks, but not double-strand breaks, was detected at each stage of purification. However, the endonuclease activity exhibited increasing dependence on disrupting virus by inclusion of 0.02% Triton X-100 at each stage of purification. At the highest stage of purification, only limited endonuclease activity was expressed without added Triton X-100, whereas significant activity was expressed in its presence (Fig. 1).

The detergent dependence of virus-associated endonuclease activity was examined further with covalent circular PM2 DNA as a substrate. In addition, viral samples purified from culture medium without serum were examined to minimize contamination of virus with endonuclease activities contained in serum. The data shown in Table 1 demonstrate that highly purified virus exhibited endonuclease activity only when disrupted by a nonionic detergent Nonidet P-40. Only limited activity was observed in nondisrupted virus, regardless of whether serum was included in the culture medium provided R-MuLV-producing cells.

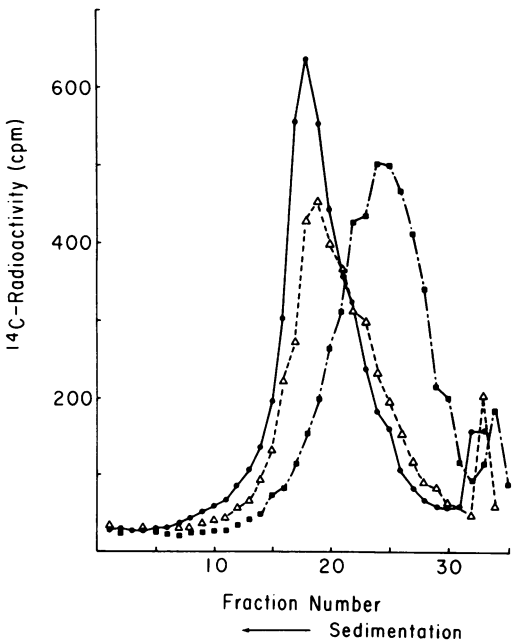


FIG. 1. *Detergent-dependent endonuclease activity. Approximately 10 μg of purified virus was incubated with about 3,000 cpm of ^{14}C-labeled T7 double-stranded DNA for 2 h at 37°C in 10 mM $MgCl_2$. The product was applied to a 4.5-ml 5 to 20% alkaline sucrose gradient containing 0.3 M NaOH, 0.7 M NaCl, and 0.01 M EDTA and centrifuged at 42,000 rpm as described in the text. Symbols: ■, virus incubated with DNA in the presence of 0.02% Triton X-100 detergent; △, virus only; ●, detergent only.*

Partial purification of the virus-associated endonuclease. To characterize the virus-associated endonuclease further, we disrupted about 30 mg of virus containing about 5×10^7 cpm of [^{3}H]methionine-labeled protein in 5% Triton X-100, clarified the extract by centrifugation at 10,000 × *g* for 10 min, and applied the supernatant to a DEAE-Sepharose column. To facilitate purification of the virus-associated endonuclease activity, we applied the ultrasensitive solution assay described by Love and Hewitt (13), which measures single-strand breaks in molecules of covalently closed circular duplex DNA from PM2 phage. The endonuclease activity was eluted from the matrix be means of a linear gradient of NaCl (Fig. 2). The gradient fractions were also assayed for reverse transcriptase, using a poly(rC):oligo(dG) primed assay. Reverse transcriptase activity eluted just ahead of the endonuclease activity. The activity peaks were pooled and dialyzed, and the radioactive proteins were analyzed by electrophoresis in an SDS-polyacrylamide gel (Fig. 3A). The endonuclease activity coincided with lanes C and D, whereas reverse transcriptase activity was found in B and C. The fractions containing endonuclease activity contained a minor polypeptide with an approximate M_r of 40,000 (termed p40), plus several other bands, including p30 and two polypeptides of about 15,000 to 18,000 M_r. The reverse transcriptase activity peak contained a band of $p80^{pol}$, as described previously, as well as p30 and lower-M_r polypeptides (18). However, since $p80^{pol}$ appeared to be present in both the endonuclease and reverse transcriptase activity fractions, it is possible that the endonuclease fraction may contain an inhibitor to the reverse transcriptase.

Treatment of the virus with detergent and 0.5 M KCl caused a more complete disruption of the virus than did detergent alone. Therefore, in a second purification attempt, the virus was disrupted in a solution containing 5% Nonidet P-40 and 0.5 M KCl. After low-speed centrifugation and dialysis, the preparation was fractionated as in Fig. 2, and a similar profile was obtained. The endonuclease activity eluted in fractions labeled B and C in Fig. 3B; the reverse

TABLE 1. *Influence of virus disruption and culture medium on R-MuLV-associated endonuclease activity*[a]

Sample	Breaks/molecule	
	Expt. 1	Expt. 2
R-MuLV	0.088	0.100
R-MuLV (disrupted)	1.258	1.153
R-MuLV (serum-free)	0.032	0.054
R-MuLV (serum-free, disrupted)	0.999	0.840
No virus	0.032	0.000

[a] Endonuclease activity was assayed by the selective denaturation solution assay as described in the text. Reaction mixtures (200 μl) contained 10 mM Tris-hydrochloride (pH 7.5), 10 mM $MgCl_2$, 10 mM $CaCl_2$, 10 μg of PM2 DNA, and 50 μg of purified R-MuLV. Incubations were for 30 min at 37°C. Serum-free R-MuLV was prepared by first growing virus-producing cells in serum-free medium for 4 h. Four-hour culture fluids were then decanted and discarded. Cells were then grown in serum-free medium for 16 h from which serum-free R-MuLV was recovered and purified. Disrupted virus samples were prepared by treatment of purified R-MuLV with 5% Nonidet P-40 for 30 min in an ice bath. Samples to be assayed for endonuclease activity were from supernatants of disrupted virus after centrifugation at 10,000 rpm for 10 min.

transcriptase activity eluted in fractions A and B. The preparation of endonuclease activity contained a major band of p40 and a band migrating just slightly slower than p30, as well as a p30 band. Much lower amounts of p30 and almost none of the 15,000 to 18,000 M_r proteins were present in the endonuclease prepared from virus treated with both detergent and high salt than were present in the endonuclease prepared from virus treated with detergent alone (cf. Fig. 3A, lanes C and D, and Fig. 3B, lanes B and C).

The pooled endonuclease activity from the second DEAE-Sepharose column, in which virus was solubilized by 0.5 M KCl treatment, was further fractionated by chromatography on CM-Sepharose (Fig. 4). The endonuclease activity peak eluted at about 0.1 M NaCl, whereas the polymerase activity eluted at about 0.25 M NaCl. The radioactive viral proteins found in the endonuclease activity peak were analyzed by SDS-polyacrylamide gel electrophoresis. A band of about 40,000 M_r with the expected mobility of p40 was detected in the fluorogram, as was an additional polypeptide migrating slightly slower than p30. Neither the p30 nor the 15,000- to 18,000-M_r proteins were detected in this preparation (data not shown). The proteins were un-

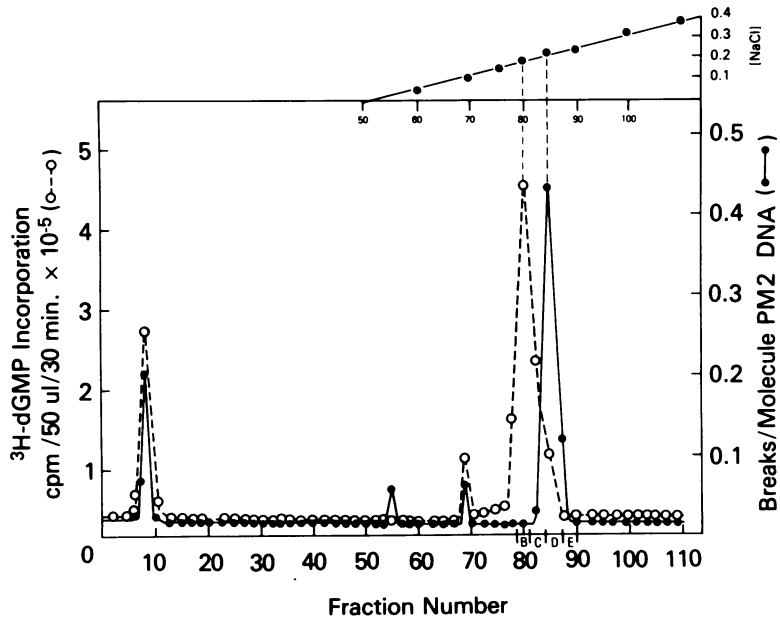


FIG. 2. *Fractionation of detergent-disrupted R-MuLV on a DEAE-Sepharose column. R-MuLV was disrupted with 5% Nonidet P-40 as described in the text and applied to a column of DEAE-Sepharose. Fractions were assayed for reverse transcriptase activity [with poly(rC):oligo(dG)] and endonuclease activity, the latter with the PM2 DNA assay (13). Fractions labeled B, C, D, and E were analyzed by SDS-polyacrylamide gel electrophoresis in Fig. 3A.*

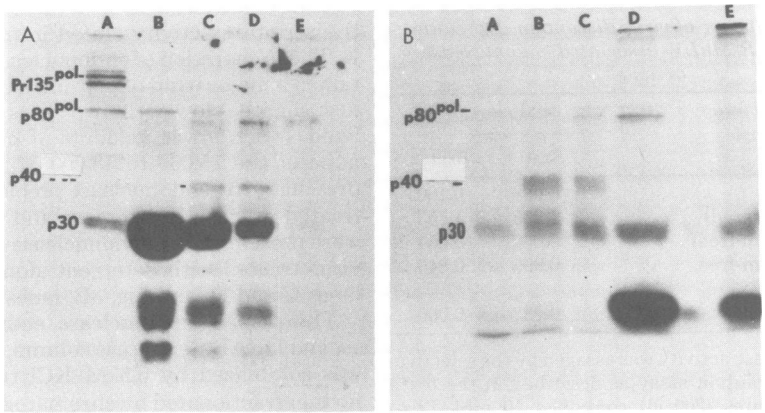


FIG. 3. *Analysis of DEAE-Sepharose column fractions by SDS-polyacrylamide gel electrophoresis. The column fractions containing reverse transcriptase and endonuclease activities were analyzed by electrophoresis on an 11.25% polyacrylamide gel containing SDS. The radioactive bands from the methionine-labeled samples were detected by fluorography. (A) DEAE-Sepharose column fractions from virus disrupted with detergent: lanes B (fractions 79–81) and C (fractions 82–84) contained the bulk of the reverse transcriptase activity; lanes C and D (fractions 85–87) contained the bulk of the endonuclease activity. (B) DEAE-Sepharose column fractions from virus disrupted with 0.5 M KCl and detergent: lanes A and B contained most of the reverse transcriptase activity; lanes B and C contained most of the endonuclease activity. The entire pooled fractions from the DEAE columns were applied to the gel in each case.*

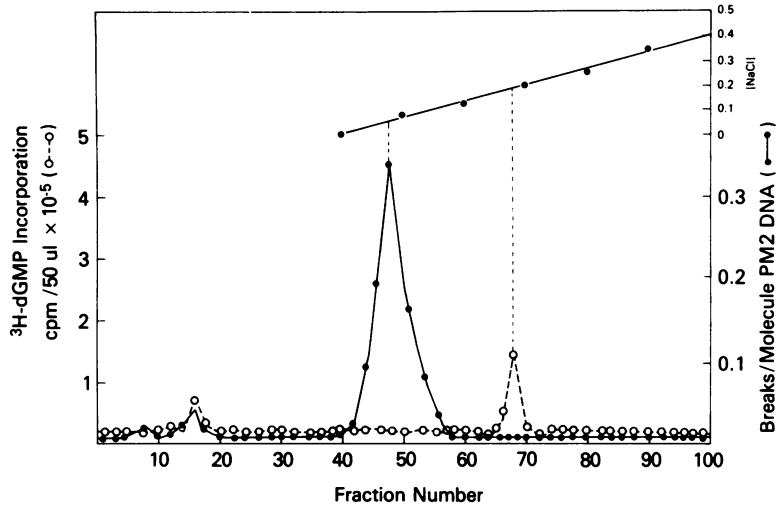


FIG. 4. *CM-Sepharose fractionation of DEAE-Sepharose column-fractionated endonuclease. Virus was disrupted with detergent in the presence of 0.5 M KCl and was clarified and fractionated on a DEAE-Sepharose column as in Fig. 2. The endonuclease peak was pooled, dialyzed, and applied to a CM-Sepharose column. The fractions were analyzed for reverse transcriptase and endonuclease activities.*

able to be analyzed by peptide mapping because of insufficient radioactivity.

The peak of endonuclease activity from the CM-Sepharose column was pooled and further fractionated by electrophoresis in a polyacrylamide gel under nondenaturing conditions (Fig. 5). A single peak of radioactive viral protein comigrated with the endonuclease activity.

The CM-Sepharose column-purified endonuclease activity did not contain detectable protease activity and RNase activity. The protease assay involved hydrolysis of radioactive casein (R. Brown, J. Kopchick, and R. Arlinghaus, unpublished data). RNase activity was assayed by testing for the ability to hydrolyze radioactive 18S and 28S rRNA (R. Bartlett, J. Kopchick,

and R. Arlinghaus, unpublished data).

Coding origin of p40. To determine whether the 40,000-molecular-weight protein associated with the endonuclease activity was a viral or host protein, we compared its peptide map with the maps of bona fide viral precursor polyproteins. Because of the yield of activity, we were forced to analyze the preparation eluting from DEAE Sepharose as in Fig. 3B. Since our earlier work showed that $Pr200^{gag\text{-}pol}$ contained additional peptide sequences not related to either *gag* or *pol* gene products (11, 12), we first compared the map of p40 with those of $Pr200^{gag\text{-}pol}$, $Pr135^{pol}$, and $p80^{pol}$. The patterns of methionine-containing tryptic peptides of these three proteins are shown in Fig. 6 and 7. In agreement with our previous results (11, 12), $p80^{pol}$ was found to share much of its peptide sequences with $Pr200^{gag\text{-}pol}$ and $Pr135^{pol}$. However, several methionine-labeled tryptic peptides derived from either $Pr200^{gag\text{-}pol}$ or $Pr135^{pol}$ were not accounted for by $p80^{pol}$ or any of the viral core proteins (Fig. 6). We emphasize that, of the four core proteins, only p30 and pp12 contain methionine (1). Furthermore, the pp12 methionine-labeled tryptic peptides do not bind to the ion-exchange resin (10).

The pattern of methionine tryptic peptides of p40 was found to contain most of these extra peptides of $Pr200^{gag\text{-}pol}$ and $Pr135^{pol}$ (Fig. 7). We have used the numbering system as published previously (12). p40 was found to contain tryptic peptides 4, 5, 7b, 11b, 12, and 13 (Fig. 7). All of these were found in $Pr200^{gag\text{-}pol}$ and $Pr135^{pol}$, except for peptide 5, which was missing from $Pr135^{pol}$. A surprising finding was that p40 contained two peptides seen in $p80^{pol}$ (no. 7b and 11b). Because of the possibility of chance coelution of nonidentical peptides, further analyses of $p80^{pol}$ and p40 need to be performed. No homology with p30 was observed. These results provide strong evidence that p40 is a virus-coded protein.

DISCUSSION

The experiments presented here provide convincing evidence that R-MuLV particles contain an endonuclease that makes single-strand breaks in either linear T7 phage, double-stranded DNA, or covalently closed circular double-stranded DNA of bacteriophage PM2. Studies on the location of the enzyme within the virus have not been done, but the fact that the detergent disruption of the virus is required for enzyme activity suggests that the endonuclease is present in the interior of the virus. Another point of interest is that this endonuclease can be separated from the reverse transcriptase activity.

An endodeoxyribonuclease has been detected in virions of Rous sarcoma virus (15) and more

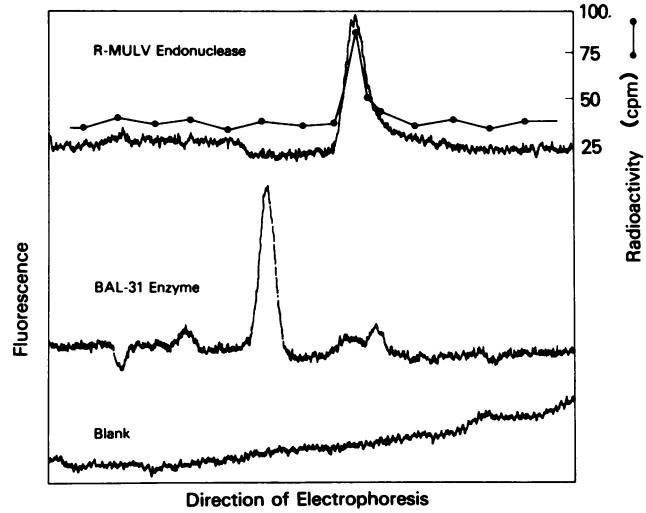
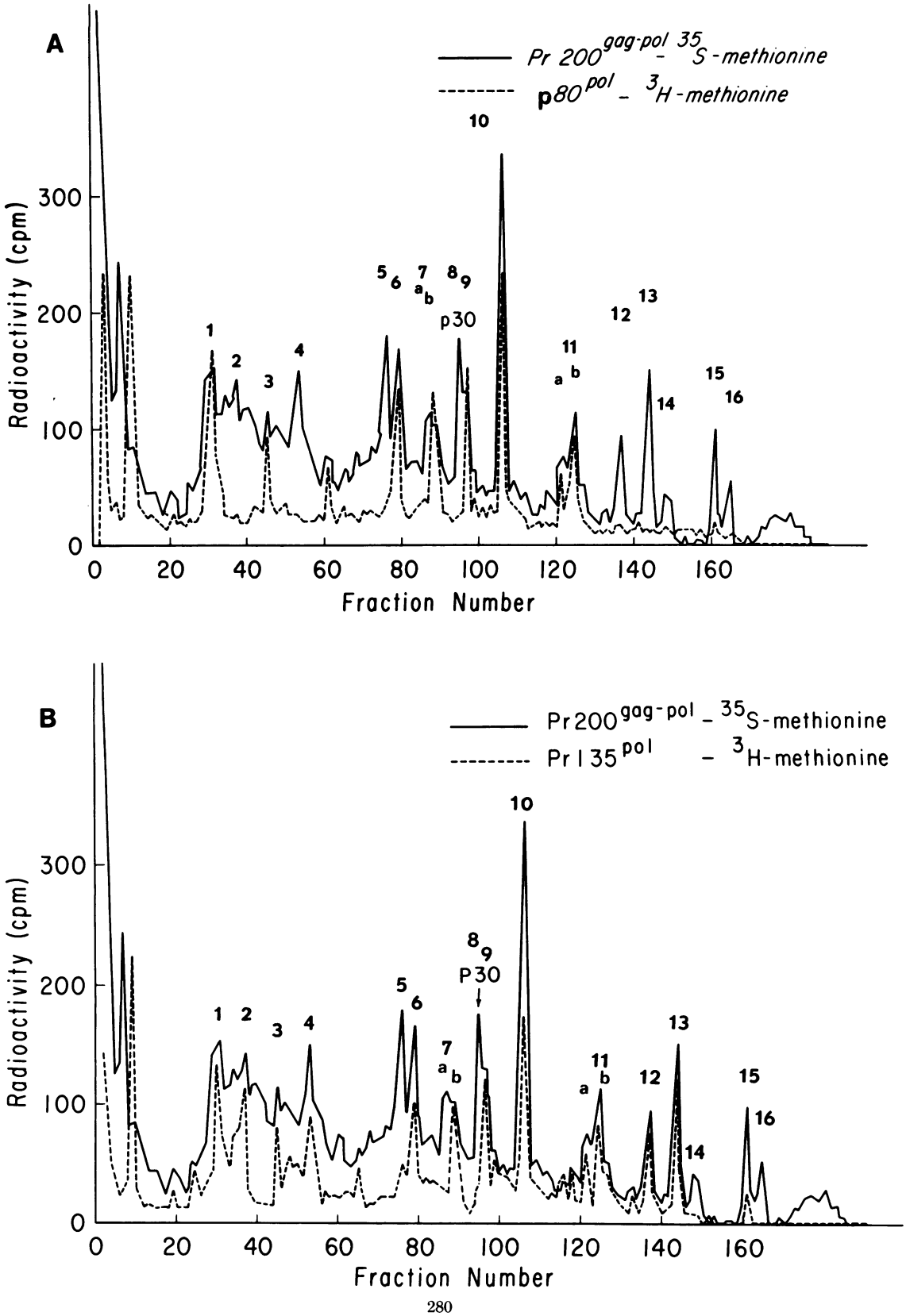


FIG. 5. *Disc gel electrophoresis of CM-Sepharose-fractionated endonuclease. (Top) The CM-Sepharose activity peak was dialyzed, and about 10 μl was applied to an 8-cm polyacrylamide gel (6-mm diameter, 7% acrylamide) containing 10 μg of closed circular PM2 DNA per ml as described (5, 13). After electrophoresis for 3.5 h at 4°C, the gel was incubated at 37°C for 60 min and then scanned for fluorescence. The endonuclease activity is identified by regions of enhanced fluorescence. The gel was then sliced, and the slices were counted in a liquid scintillation cocktail that efficiently detects radioactive protein (17). (Middle) The activity of purified BAL-31 endonuclease (4), a single-strand specific nuclease which recognizes localized regions of base-pair disruption in superhelical PM2 DNA, was included as control. (Bottom) minus enzyme analysis.*


A
Pr 200 gag-pol 35 S-methionine
p80 pol - 3 H-methionine
Radioactivity (cpm)
Fraction Number
p30
B
Pr200 gag-pol - 35 S-methionine
Pr135 pol - 3 H-methionine
Radioactivity (cpm)
Fraction Number
P30

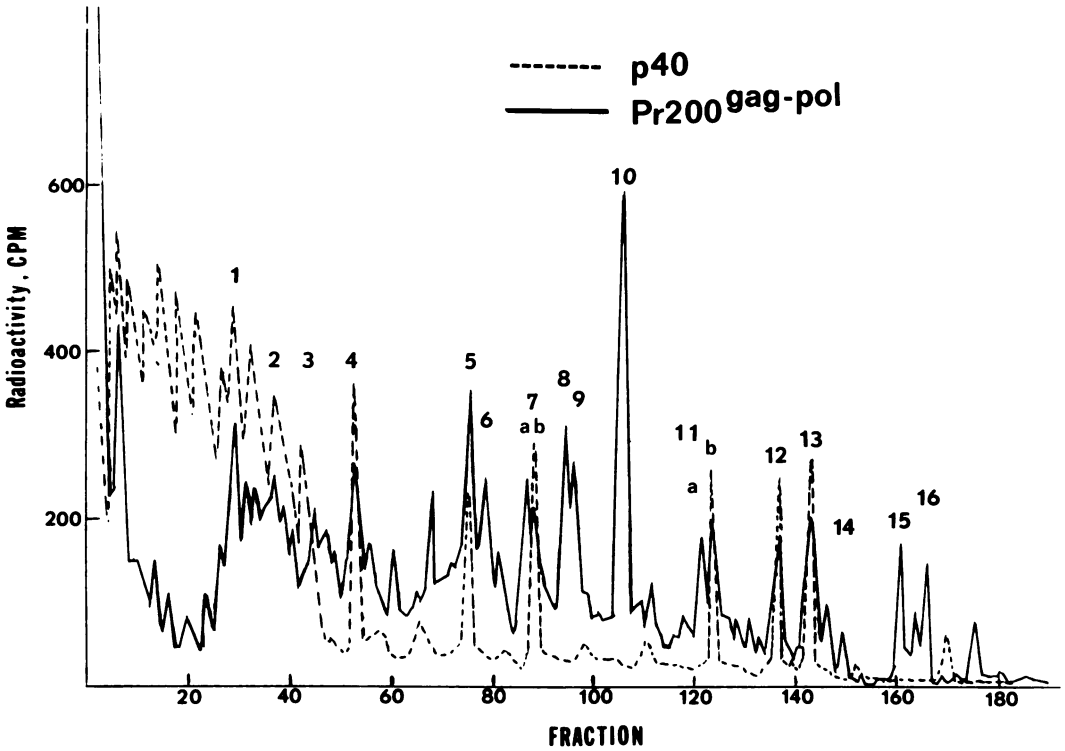


FIG. 7. *Cation-exchange chromatography of tryptic peptides of endonuclease-associated p40. [^{3}H]methionine-labeled p40 isolated by DEAE-Sepharose chromatography and SDS-polyacrylamide gel electrophoresis (as in Fig. 3B) was digested with tolylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-trypsin, and the soluble tryptic peptides were fractioned on cation-exchange column (1). [^{35}S]methionine Pr200$^{gag\text{-}pol}$ was isolated from R-MuLV-infected NIH Swiss mouse cells as described (11). Pr200$^{gag\text{-}pol}$ was digested with TPCK-trypsin and added as marker. The methionine-tryptic peptides have been arbitrarily numbered as described previously (12).*

recently has been isolated and purified from avian myeloblastosis virus particles (2, 3, 23). In contrast to the results presented here, the avian endonuclease appears to be part of the reverse transcriptase enzyme complex. The avian reverse transcriptase is composed of two polypeptides, one of 95,000 M_r termed the β subunit and one of 65,000 M_r termed the α subunit (15, 23). A 32,000-molecular-weight fragment of the C terminal third of the β subunit contains the endonuclease activity (2, 3, 23).

The subunit structure of the MuLV-associated endonuclease is not yet known. Our results indicate that an approximately 40,000-M_r viral polypeptide (p40) is associated with the partially purified enzymatic activity. Our tryptic peptide analyses have shown that p40 shares at least six major methionine-containing tryptic peptides (nos. 4, 5, 7b, 11b, 12, and 13) with Pr200$^{gag\text{-}pol}$ the precursor to the reverse transcriptase (p80^{pol}; 8, 11, 12). Also, p40 was found to share five methionine-containing tryptic peptides with Pr135^{pol} (nos. 4, 7b, 11b, 12, and 13). The murine p80^{pol} and p40 appear to be quite different in structure except for two tryptic peptide fractions (nos. 7b and 11b), which may be just chance comigration of nonidentical peptides.

Proof that p40 is, in fact, the virus-coded endonuclease awaits further work, but several considerations favor such an interpretation.

FIG. 6. *Cation-exchange profiles of methionine-containing tryptic peptides of p80^{pol} and Pr135^{pol}. (A) [^{3}H]methionine-labeled p80^{pol} and [^{35}S]methionine-labeled Pr200$^{gag\text{-}pol}$ were isolated from R-MuLV-infected JLS-V16 cells by anti-reverse transcriptase immunoprecipitation and SDS-polyacrylamide gel electrophoresis as described (11, 12). Samples of tryptic digests were chromatographed on a cation-exchange column as described (1). The methionine-tryptic peptides have been arbitrarily numbered as described previously (12). (B) [^{3}H]methionine-labeled Pr135^{pol} and [^{35}S]methionine-labeled Pr200$^{gag\text{-}pol}$ were isolated and digested with trypsin as in (A).*

First, p40 was always detected in partially purified endonuclease preparations, including enzyme preparations that were fractionated first on DEAE-Sepharose and then CM-Sepharose. Second, p40 has a similar size to the 32,000-M_r endonuclease of the avian retroviruses. Third, the precursor origin and mode of formation of p40 appear to be similar to the avian virus-coded endonuclease. Studies performed by Grandgenett and co-workers have shown that the avian endonuclease (p32) apparently is generated by cleavage of the β subunit of the reverse transcriptase (2, 3, 23). The β subunit, itself, is produced by cleavage of the avian *gag-pol* gene product, Pr180 (21, 22). A similar pathway seems likely for the formation of p40. Thus, p40 appears to be derived from $Pr200^{gag\text{-}pol}$ by way of $Pr135^{pol}$; the latter appears to be the murine retrovirus analog of the β subunit. Of course, a major difference exists between the two virus systems since $Pr135^{pol}$ does not appear to be part of the MuLV reverse transcriptase enzyme (16, 26).

We have previously proposed a model that states that $Pr200^{gag\text{-}pol}$ is the precursor to $p80^{pol}$, plus other enzymes (12). We view $Pr200^{gag\text{-}pol}$ as not only the precursor to viral enzymes, but also as providing a mechanism for packaging the virus-coded enzymes into the infectious particle. We propose that the *gag* gene-coded portion of $Pr200^{gag\text{-}pol}$ permits virus-coded enzymes that are contained within the precursor to be packaged into viral particles by virtue of its p10 and p12 sequences. These sequences favor selective binding to viral RNA (7).

The role of a virus-coded endonuclease in retrovirus replication is not yet known, but it is obvious that such an enzyme could be involved in integration of viral DNA by making staggered single-strand cuts in the double-stranded DNA of the host and in viral DNA intermediates generated during the reverse transcriptase reaction.

ACKNOWLEDGMENTS

This work was supported by grants from the Robert A. Welch Foundation (G-429), the National Cancer Institute (CA-25465), and core grant (CA-16672). J.J.K. was a predoctoral fellow supported by the Rosaile B. Hite Foundation.

We thank Nancy Nickels and James Syrewicz for technical assistance and Rebecca Bazer for assistance in manuscript preparation.

LITERATURE CITED

1. **Arcement, L. J., W. L. Karshin, R. B. Naso, and R. B. Arlinghaus.** 1977. 'gag' polyprotein precursors of Rauscher murine leukemia virus. Virology **81**:284-297.
2. **Grandgenett, D. P., M. Golomb, and A. C. Vora.** 1980. Activation of an Mg^{2+}-dependent DNA endonuclease of avian myeloblastosis virus $\alpha\beta$ DNA polymerase by in vitro proteolytic cleavage. J. Virol. **33**:264-271.
3. **Grandgenett, D. P., A. C. Vora, and R. D. Schiff.** 1978. A 32,000 dalton nucleic acid-binding protein from avian retrovirus cores possesses DNA endonuclease activity. Virology **89**:119-132.
4. **Gray, H., Jr., D. Ostrander, J. Hodnett, R. Legerski, and D. Robberson.** 1975. Extracellular nucleases of *Pseudomonas* BAL-31. I. Characterization of single strand specific deoxyriboendonuclease and double strand deoxyriboexonuclease activities. Nucleic Acids Res. **2**:1459-1492.
5. **Grdina, D. J., P. H. Lohman, and R. R. Hewitt.** 1973. A fluorometric method for the detection of endodeoxyribonuclease on DNA-polyacrylamide gels. Anal. Biochem. **51**:255-264.
6. **Hewitt, R., J. C. Suit, and D. Billen.** 1967. Utilization of 5-bromouracil by thymineless bacteria. J. Bacteriol. **93**:86-89.
7. **Jamjoom, G. A., R. B. Naso, and R. B. Arlinghaus.** 1976. A selective decrease in the rate of cleavage of an intracellular precursor to Rauscher leukemia virus p30 by treatment of infected cells with actinomycin D. J. Virol. **19**:1054-1072.
8. **Jamjoom, G. A., R. B. Naso, and R. B. Arlinghaus.** 1977. Further characterization of intracellular precursor polyproteins of Rauscher leukemia virus. Virology **78**: 11-34.
9. **Kacian, D. L., K. F. Watson, A. Burny, and S. Spiegelman.** 1971. Purification of the DNA polymerase of avian myeloblastosis virus. Biochim. Biophys. Acta **246**:365-383.
10. **Karshin, W. L., L. J. Arcement, R. B. Naso, and R. B. Arlinghaus.** 1977. Common precursor for Rauscher leukemia virus gp69/71, p15(E), and p12(E). J. Virol. **23**:787-798.
11. **Kopchick, J. J., G. A. Jamjoom, K. F. Watson, and R. B. Arlinghaus.** 1978. Biosynthesis of Rauscher murine leukemia virus reverse transcriptase by cleavage of a 'gag-pol' read-through precursor polyprotein. Proc. Natl. Acad. Sci. U.S.A. **75**:2016-2020.
12. **Kopchick, J. J., W. L. Karshin, and R. B. Arlinghaus.** 1979. Tryptic peptide analysis of 'gag' and 'gag-pol' gene products of Rauscher murine leukemia virus. J. Virol. **30**:610-623.
13. **Love, J. D., and R. R. Hewitt.** 1979. The relationship between human serum and human pancreatic DNase I. J. Biol. Chem. **254**:12588-12594.
14. **McDonell, M., M. Simm, and F. Studier.** 1977. Analyses of restriction fragments of T7 DNA and determination of mol. wts. by electrophoresis in neutral and alkaline gels. J. Mol. Biol. **110**:119-146.
15. **Mitzutani, S., D. Boettiger, and H. M. Temin.** 1970. A DNA-dependent DNA polymerase and a DNA endonuclease in virions of Rous sarcoma virus. Nature (London) New Biol. **228**:424-427.
16. **Moelling, K.** 1976. Further characterization of the Friend murine leukemia virus reverse transcriptase-RNase H complex. J. Virol. **18**:418-425.
17. **Murphy, E. C., Jr., and R. B. Arlinghaus.** 1980. Effect of canavanine on murine retrovirus polypeptide formation. J. Virol. **33**:954-961.
18. **Murphy, E. C., Jr., J. J. Kopchick, K. F. Watson, and R. B. Arlinghaus.** 1978. Cell-free synthesis of a precursor polyprotein containing both 'gag' and 'pol' gene products by Rauscher murine leukemia virus 35S RNA. Cell **13**:359-369.
19. **Naso, R. B., L. J. Arcement, and R. B. Arlinghaus.** 1975. Biosynthesis of Rauscher leukemia viral proteins. Cell **4**:31-36.
20. **Naso, R. B., W. L. Karshin, Y. H. Wu, and R. B. Arlinghaus.** 1979. Characterization of 40,000- and 25,000-dalton intermediate precursors to Rauscher murine leukemia virus '*gag*' gene products. J. Virol. **32**: 187-198.

21. **Oppermann, H., J. M. Bishop, H. E. Varmus, and L. Levintow.** 1977. A joint product of the genes 'gag' and 'pol' of avian sarcoma virus: a possible precursor of reverse transcriptase. Cell **12**:933–1005.
22. **Paterson, B., D. J. Marciani, and T. S. Papas.** 1977. Cell-free synthesis of the precursor polypeptide for avian myeloblastosis virus DNA polymerase. Proc. Natl. Acad. Sci. U.S.A. **74**:4951–4954.
23. **Schiff, R. D., and D. P. Grandgenett.** 1978. Virus-coded origin of a 32,000-dalton protein from avian retrovirus cores: structural relatedness of p32 and the β polypeptide of the avian retrovirus DNA polymerase. J. Virol. **28**:279–291.
24. **Syrewicz, J. J., R. B. Naso, C. S. Wang, and R. B. Arlinghaus.** 1972. Purification of large amounts of murine RNA tumor viruses produced in roller bottle cultures. Appl. Microbiol. **24**:488–498.
25. **Thomas, C. A., Jr., and J. Abelson.** 1966. The isolation and characterization of DNA from bacteriophage, *In* p. 553–568. G. L. Cantoni and D. R. Davies (ed.), Procedures in Nucleic Acid Research. Harper and Row, New York.
26. **Verma, J.** 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.