Radioimmunoassay for Mammalian Type C Viral Reverse Transcriptase

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Radioimmunological techniques were applied to the analysis of reverse transcriptases of mammalian type C RNA viruses. The polymerase of Rauscher mouse leukemia virus was purified by ion exchange and sequential affinity chromatography. Radioimmunoassays that utilized the viral enzyme as a probe detected as little as 1 ng of purified polymerase. No cross-reactivity could be demonstrated between the reverse transcriptase and other known virus-coded proteins. By comparing the immunological reactivity of the purified enzyme with the reactivity of detergent-disrupted virions, Rauscher mouse leukemia virus was shown to contain the antigenic equivalent of 40 molecules of reverse transcriptase. In a homologous competition immunoassay, the Rauscher viral enzyme demonstrated type-specific antigenic determinants, which distinguish it from other mouse type C viral polymerases. In a broadly reactive interspecies immunoassay, the reverse transcriptases of a number of mammalian type C viruses were cross-reactive, indicating their shared antigenic determinants. Various treatments that inhibited or activated DNA polymerase activity had little or no effect on the immunological properties of the enzyme. Thus, radioimmunoassays should be useful in the search for type C viral reverse transcriptase as a marker of subviral expression.

Reverse transcriptases of mammalian type C RNA viruses have been purified and characterized biochemically (4, 7, 20, 39, 46). The usefulness of immunological approaches in the identification and classification of these enzymes was initially indicated by observations that antisera to partially purified mouse viral polymerase specifically inhibited mammalian type C viral, as opposed to non-type C viral or host, cellular polymerases (1, 2, 31). Polymerase inhibition studies, utilizing antisera prepared against a variety of mammalian type C viral polymerases, have been extended to indicate immunological cross-reactivity among the enzymes of different groups of mammalian type C viruses (27, 33). By this technique, it has not been possible to discriminate among polymerases of closely related viruses or to demonstrate broadly shared antigenic determinants among the known mammalian type C viral enzymes. Moreover, since this assay is based upon detection of enzyme activity it is dependent upon the presence of functional reverse transcriptase.

The development of competition radioimmunoassays has provided a valuable method for studying the immunological properties of mammalian type C viral structural proteins. This technique has proved useful in detecting endogenous viral antigen expression in the absence of virus release (28, 35), in defining the immunological relatedness of different viral isolates (12, 37, 42), and in mapping the viral genome (5). A radioimmunoassay has recently been reported for avian type C viral reverse transcriptase (25). In the present study, radioimmunological techniques have been applied to the characterization of Rauscher mouse leukemia virus (MuLV) reverse transcriptase. The immunological properties of this viral enzyme are compared with those of other mammalian type C viruses.

MATERIALS AND METHODS

Viruses. Mouse type C viruses included Rauscher (29), Moloney (22), and Gross (11) leukemia viruses and BALB virus-2, an endogenous xenotropic virus of the BALB/c strain (3). Other viruses analyzed included the Rickard strain of feline leukemia virus (30), RD114, an endogenous virus of cat cells (18), and a type C virus isolated from the woolly monkey (40). Mouse mammary tumor virus (17) and bovine leukemia virus (44) were also tested. Viruses were obtained as sucrose gradient-purified preparations from Electro-Nucleonics, Rockville, Md., Pfizer Laboratories, Maywood, N.J., or Frederick Cancer Research Center, Frederick, Md., through the courtesy of J. Gruber, Office of Resources and Logistics, National Cancer Institute (NCI).

Antisera. Antisera to the partially purified re-
verse transcripts of Rauscher MuLV (27), wooly monkey virus, and avian myeloblastosis virus, as well as antisera to Rauscher MuLV p30, p15, p12, p10, and gp70, were of caprine origin and generously supplied by R. Wilsnack, Huntington Research Laboratories, through the Office of Resources and Logistica, NCI. Guinea pig anti-RD114 reverse transcriptase was a gift of R. Gilden, Frederick Cancer Research Center.

Purification of Rauscher MuLV reverse transcriptase. Rauscher MuLV (102 mg of protein) was centrifuged for 90 min at 100,000 × g. The resulting pellet was resuspended in 5 ml of buffer containing 10 mM Tris, pH 7.8, 500 mM KCl, 1 mM dithiothreitol (DTT), 20% glycerol, and 1% Triton X-100. After incubation for 2 h, an equal volume of buffer A (10 mM Tris, pH 8.0, 25 mM KCl, 1 mM DTT, 20% glycerol, and 0.1% Triton) was added, and the disrupted virus was dialyzed overnight against 200 volumes of buffer A. This and all subsequent steps were carried out at 4°C. The dialyzed preparation was applied to a DEAE-cellulose (Whatman DE-52) column (1.0 by 6.0 cm) previously equilibrated with buffer A. After it had been washed with 25 ml of the same buffer, the sample was eluted with an 80-ml linear, 25 to 500 mM KCl gradient in buffer A. Figure 1A shows the elution profile of enzyme activity from DEAE-cellulose. Fractions with enzyme activity eluting between 40 and 85 mM KCl were pooled, dialyzed overnight against 200 volumes of buffer B (10 mM Tris, pH 7.2, 50 mM KCl, 1 mM DTT, 20% glycerol, 0.1% Triton, and 0.1 mM MnCl2), and further purified by sequential affinity chromatography. The partially purified enzyme was applied to a column (0.8 by 5.0 cm) of oligo(deoxyribosylthymidine)12-18-oligo(dT)12-18-cellulose (Collaborative Research) previously equilibrated with buffer B. After a 20-ml wash, the sample was eluted with an 80-ml linear, 50 to 1,000 mM KCl gradient in the same buffer. Fractions with peak enzyme activity, eluting between 170 and 205 mM KCl (Fig. 1B), were pooled and dialyzed overnight against 200 volumes of buffer C (10 mM Tris, pH 7.8, 100 mM KCl, 1 mM DTT, 20% glycerol, 0.1% Triton, and 0.1 mM MnCl2). Polyadenosine acid [poly(A)]-oligo(dT)12-18-cellulose was prepared as previously described (9) and packed into a column (0.8 by 5.0 cm). The column was washed with 20 ml of buffer C with 1 M KCl and 80 ml of buffer C with 0.1 M KCl at 4°C. The partially purified enzyme was applied, and the column was washed with 20 ml of buffer C containing 0.1 M KCl, and eluted with an 80-ml linear, 0.1 to 1.0 M KCl gradient in the same buffer. Fractions containing peak enzyme activity, eluting between 225 and 265 mM KCl (Fig. 1C), were shown to contain a single polypeptide of 80,000 daltons by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. Fractions were stored individually at −70°C, and only the peak fraction was further utilized for radioimmunological studies.

DNA polymerase assays. Enzymatic activity was determined in a 0.1-ml reaction mixture consisting of 50 mM Tris, pH 7.8, 90 mM KCl, 2 mM DTT, 0.05% Triton, 0.1 mM MnCl2, 500 ng of poly(rA), 375 ng of oligo(dT)12-18, and 400 pmol of [3H]TTP (50 Ci/mmole). DNA synthesis, measured as acid-precipitable radioactivity after incubation for 1 h at 37°C, was determined as previously described (31). The ability of antisera to inhibit enzymatic activity was measured by adding 10 μl of appropriate dilutions of the antisera to be tested to the reaction mixture. For inhibition studies, the amount of enzyme added was standardized to incorporate 25 pmol of [3H]TMP into acid-precipitable material in 1 h at 37°C in the absence of antisera.

RNase H assays were performed as previously described (7). PAGE. PAGE, in the presence of 0.1% SDS (SDS-PAGE), was performed according to the method of Laemmli (13). Acrylamide gels (10%) were stained with Coomassie blue; gels containing radiolabeled proteins were frozen, cut into 1-mm sections, and analyzed for radioactivity in a gamma counter (Searle model 1285).

Protein determinations. Protein was determined by the method of Lowry et al. (16), with bovine serum albumin as a standard. In highly purified enzyme preparations, fractions to be used for protein determinations were lyophilized and concentrated 20-fold before protein assays. In some cases, independent estimates of protein concentrations were obtained by Coomassie blue staining in 10% SDS-PAGE gels: staining density of the enzyme preparation was compared with that of known quantities of

![Figure 1. Purification of Rauscher MuLV reverse transcriptase by ion exchange and sequential affinity chromatography. Fractions were analyzed for DNA polymerase activity using poly(A)-oligo(dT)12-18 as template in the presence of 50 mM Tris, pH 7.8, 90 mM KCl, 0.1 mM MnCl2, 0.05% Triton, 2 mM DTT, and 400 pmol of [3H]TTP. Salt concentrations were determined conductimetrically. (A) DEAE-cellulose chromatography; (B) oligo(dT)12-18-cellulose chromatography; (C) poly(A)-oligo(dT)12-18-cellulose chromatography.](http://jvi.asm.org/content/pdfs/3/JV101062F1.pdf)
phosphorylase A and bovine serum albumin. Protein determinations by both methods were comparable.

Radioimmunoassay for reverse transcriptase. Purified enzyme was labeled with \(^{125}\text{I}\) by the method of Greenwood et al. (10). Reaction mixtures (70 \(\mu\)l) consisted of 0.6 \(\mu\)g of reverse transcriptase, 1.0 mCi of \(^{125}\text{I}\) (100 mCi/\(\mu\)g, Amersham/Searle), and 25 \(\mu\)g of chloramine T in a buffer consisting of 50 mM Tris, pH 7.8, and 100 mM KCl. After 1 min at 25\(^\circ\)C, the reaction was terminated by the addition of 60 \(\mu\)g of sodium metabisulfite. The \(^{125}\text{I}\)-labeled protein was separated from free \(^{125}\text{I}\) by gel filtration (P-10 polyclaramide gel, Bio-Rad) in a column (0.3 by 10 cm) equilibrated and eluted with 10 mM Tris, pH 7.8, and 100 mM KCl. Iodinated protein (33 \(\mu\)Ci/\(\mu\)g) was diluted and stored at –70\(^\circ\)C in buffer D (10 mM Tris, pH 7.8, 100 mM KCl, 10\% glycerol, 0.1\% Triton, 0.5\% bovine serum albumin, and 0.05\% sodium azide). Double antibody immunoprecipitation and competition radioimmunoassays were performed as previously described (12), except that all reagents were prepared or diluted in buffer D.

RESULTS

Purification of Rauscher MuLV reverse transcriptase. In developing a radioimmunoassay for the reverse transcriptase of Rauscher MuLV, an attempt was made to utilize purification procedures that would yield highly purified enzyme with native antigenicity. Recent evidence has indicated that the viral polymerase can be degraded during prolonged purification procedures (21). These findings suggested the use of affinity chromatography, which could be performed rapidly and under conditions similar to those required for optimal enzyme activity (7-9). Chromatography on DEAE-cellulose was used as the initial purification step because of its usefulness in removing contaminating nucleic acids that might alter the binding of reverse transcriptase to affinity chromatography substrates. Furthermore, under the conditions employed, most contaminating viral structural proteins eluted in the wash (data not shown). Table 1 summarizes the results of viral enzyme purification.

When reverse transcriptase, partially purified by ion exchange chromatography, was applied to an oligo(dT)\(_{12-18}\)-cellulose affinity column, 95\% of the enzymatic activity was bound to the substrate and eluted in a single peak. However, SDS-PAGE analysis of these peak fractions revealed not only a major polypeptide of 80,000 molecular weight, but additional polypeptides of 60,000 to 70,000 molecular weight. This affinity chromatography step yielded a further threefold purification of enzymatic activity.

The addition of a second affinity chromatography step, poly(A)·oligo(dT)\(_{12-18}\)-cellulose, resulted in a further 21-fold purification of reverse transcriptase for a final purification of over 170-fold (Table 1). The purified protein was shown to possess RNA-dependent DNA polymerase and RNase H activities. SDS-PAGE analysis of the fractions containing peak reverse transcriptase activity revealed only a single polypeptide of 80,000 daltons. Reverse transcriptase, purified by ion exchange and sequential affinity chromatography, has remained stable for over 4 months at –70\(^\circ\)C.

Characterization of \(^{125}\text{I}\)-labeled Rauscher MuLV reverse transcriptase. To prepare a radiolabeled probe for immunosassay, the purified Rauscher MuLV polymerase was iodinated by the chloramine T method. As shown in Fig. 2, the iodinated product migrated as a single band of 80,000 molecular weight by SDS-PAGE and was judged to be more than 90\% radiochemically pure by electrophoretic criteria. To further establish the identity of the \(^{125}\text{I}\)-labeled protein, purified, enzymatically active Rauscher MuLV reverse transcriptase and the labeled

**Table 1. Purification of Rauscher-MuLV reverse transcriptase**

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total protein* (mg)</th>
<th>Total enzymatic activity* (pmol of TMP incorporated (\times 10^{10}) *)</th>
<th>Sp act (pmol of TMP incorporated (\times 10^{-6})/mg of protein)</th>
<th>Purification (-fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Disruption</td>
<td>102.0</td>
<td>32.0</td>
<td>0.3</td>
<td>1</td>
</tr>
<tr>
<td>(2) DEAE-cellulose chromatography</td>
<td>17.0</td>
<td>16.0</td>
<td>0.9</td>
<td>3</td>
</tr>
<tr>
<td>(3) Oligo(dT)(_{12-18})-cellulose chromatography</td>
<td>1.8</td>
<td>4.5</td>
<td>2.5</td>
<td>8</td>
</tr>
<tr>
<td>(4) Poly(A)·oligo(dT)(_{12-18})-cellulose chromatography</td>
<td>0.07</td>
<td>3.6</td>
<td>51.0</td>
<td>172</td>
</tr>
</tbody>
</table>

* Viral enzyme purification was performed as described in Materials and Methods.

* Protein was determined by the method of Lowry et al. (16). Fractions with peak enzyme activity were pooled at each purification step except for the last, where protein in the single fraction containing peak activity was measured.

* For determination of enzymatic activity, aliquots of pooled fractions at each purification step were analyzed for ability to catalyze the incorporation of \(^3\text{H}\)/TMP into acid-precipitable material in a standard DNA polymerase assay as described in the text.
probe were submitted to gel filtration chromatography under denaturing conditions (Fig. 3). The enzymatic activity and labeled probe chromatographed, eluting at a position corresponding to a molecular weight of around 80,000. These findings substantiate that the labeled probe was 125I-labeled Rauscher MuLV reverse transcriptase.

Radioimmunoassay for Rauscher MuLV reverse transcriptase. The ability of various antisera to precipitate the 125I-labeled reverse transcriptase was next examined. As shown in Fig. 4, more than 95% of the labeled enzyme was precipitated by an antiserum prepared against partially purified Rauscher MuLV reverse transcriptase, demonstrating the preservation of the immunological properties of the enzyme during the 125I-labeling procedure. Antisera prepared against the polymerases of woolly monkey virus and RD114 also precipitated the radiolabeled enzyme, although at lower titers. As controls, antisera raised against other Rauscher MuLV proteins, gp70, p30, p15, p12, and p10, did not detectably precipitate the 125I-labeled reverse transcriptase, although each antiserum was able to precipitate its respective 125I-labeled protein at a titer of 1:10,000 or greater (data not shown). These results demonstrate that the reverse transcriptase possesses antigenic determinants not shared by other known viral proteins.

A competition radioimmunoassay was developed that utilized 125I-labeled Rauscher MuLV reverse transcriptase and limiting amounts of antiserum directed against the homologous enzyme. As shown in Fig. 5, as little as 1 ng of purified viral enzyme was detectable. Detergent-disrupted Rauscher MuLV, grown in either mouse or rat cells, was also able to compete fully in the assay. The fact that the same virus grown in cells of different species showed identical immunoreactivity indicates the virus specificity of the assay.

In comparing the competition curves for purified enzyme and disrupted virions, Rauscher MuLV was about 250-fold less efficient than purified enzyme on a weight basis in competing for binding sites with the 125I-labeled probe.
Assuming that the immunoreactivity of the enzyme was neither enhanced nor diminished during purification, approximately 0.4% of the protein in the disrupted viral preparation would be reverse transcriptase. This corresponds to the antigenic equivalent of about 40 molecules of reverse transcriptase in the average virion.

Antigenic determinants of Rauscher MuLV reverse transcriptase: type, group, and interspecies reactivities. Known mammalian type C viral proteins contain a range of antigenic determinants that vary in their degree of cross-reactivity with analogous proteins of viruses of the same or different species. In the homologous immunooassay for Rauscher MuLV polymerase, three patterns of reactivity were observed among the viruses tested. As shown in Fig. 6, only Rauscher MuLV competed fully with labeled Rauscher MuLV polymerase for antisemur-binding sites. Other mouse type C viruses, including Gross MuLV, Moloney MuLV, and BALB virus-2, showed only partial competition. Their competition curves were relatively shallow and plateaued at about 50% displacement of the labeled probe. A third group, comprising nonmurine mammalian type C viruses, was unable to compete in this assay. These results indicated that the Rauscher viral polymerase contained type-specific antigenic determinants, as well as determinants shared with other mouse viral polymerases. It should be noted that when the same anti-Rauscher MuLV reverse transcriptase serum was used to directly inhibit viral enzyme activities, Rauscher MuLV could not be distinguished from the other mouse type C viruses tested (Fig. 7).

The polymerases of mammalian type C viruses have been classified into several related groups on the basis of polymerase inhibition studies (27, 33). The reactivities of different mammalian type C viruses were tested in a heterologous competition immunoassay using limiting amounts of antisemur to woolly monkey viral polymerase to precipitate 125I-labeled Rauscher viral reverse transcriptase. As shown in Fig. 8, all mouse type C viruses tested competed fully in this assay. Further, type C vi-
pmol of 336
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FIG. 6. Detection of type-specific determinants of Rauscher MuLV reverse transcriptase. Serial two- fold dilutions of detergent-disrupted viruses were tested for their ability to compete in the homologous immunoassay utilizing limiting antisera to Rauscher MuLV reverse transcriptase to precipitate 125I-labeled Rauscher viral polymerase. Viruses tested included: Rauscher MuLV (○), Gross MuLV (△), Moloney MuLV (▲), BALB virus-2 (▼), wooly monkey virus (□), RD114 (●), feline leukemia virus (●), and avian myeloblastosis virus (▲).

FIG. 7. Inability of direct polymerase inhibition to distinguish among the mouse type C virus reverse transcriptases. Inhibition of reverse transcriptase activity by anti-Rauscher reverse transcriptase was measured by adding the indicated amounts of antisem to standard DNA polymerase reaction mixtures (see text) catalyzed by enzyme derived from Rauscher MuLV (○), Gross MuLV (△), Moloney MuLV (▲), and BALB virus-2 (▼). The amount of enzyme added to each reaction was standardized to incorporate 25 pmol of [3H]TMP into acid-precipitable material in the absence of inhibiting antisem under the conditions described in the text. The effect of preimmune goat serum on polymerase activity of Rauscher MuLV is also shown (●).

FIG. 8. Shared interspecies antigenic determinants of mammalian type C viral reverse transcriptases detected in a heterologous immunoassay. Serial twofold dilutions of detergent-disrupted viruses were tested for their ability to compete with 125I-labeled Rauscher reverse transcriptase for limiting amounts of anti-woolly monkey virus reverse transcriptase. Type C viruses included Rauscher-MuLV (●), Gross MuLV (△), Moloney MuLV (▲), BALB virus-2 (▼), wooly monkey virus (□), RD114 (●), and feline leukemia virus (○). Other reverse transcriptase containing viruses tested were syncytium-forming (foamy) virus (○), bovine leukemia virus (●), mouse mammary tumor virus (□), and avian myeloblastosis virus (▲).

The enzymes of other species, including wooly monkey virus, RD114, and feline leukemia virus, were able to completely displace the labeled probe. Thus, the heterologous assay detected antigenic determinants shared among reverse transcriptases of all mammalian type C viruses tested. In contrast, an avian type C virus and mammalian non-type C viruses with reverse transcriptases, including MTV, bovine leukemia virus, and syncytium-forming (foamy) virus, failed to compete. The polymerases of these latter viruses, although functionally similar to those of mammalian type C viruses, did not demonstrate shared immunological determinants detectable in this assay.

Detection of immunoreactive reverse transcriptase in the absence of enzyme activity. A major limitation of assays for reverse transcriptase based upon enzymological activity is the inability to detect inactive enzyme or enzyme in the presence of inhibitors. To determine the ability of the radioimmunoassay to measure reverse transcriptase under such conditions, the immunoreactivity of the enzyme was measured after various treatments that caused in-
hibitation or inactivation of enzyme activity.

As shown in Table 2, purified Rauscher MuLV reverse transcriptase or enzyme from disrupted Rauscher MuLV was markedly inhibited by the addition of EDTA or sodium pyrophosphate. However, when the enzyme was assayed for immunological reactivity in the presence of either of these inhibitors, there was no loss of antigenicity. The enzyme was also inactivated by a variety of physical (heating, lyophilization, and denaturation in 6 M guanidine-hydrochloride) or chemical (oxidation by chloramine T and alkylation by N-ethylmaleimide) methods. Each of these treatments resulted in at least 95% loss of enzyme activity. In contrast, little, if any, alteration in the immunoreactivity of either purified enzyme or enzyme from disrupted virions was detected after any of these treatments (Table 2).

**Table 2. Enzymatic and immunological activity of viral reverse transcriptase after treatment with enzyme inhibitors and inactivators**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activity remaining after treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disrupted Rauscher-MuLV</td>
</tr>
<tr>
<td></td>
<td>Enzymatic</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>10 mM EDTA</td>
<td>&lt;1</td>
</tr>
<tr>
<td>10 mM pyrophosphate</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Lyophilization</td>
<td>NT</td>
</tr>
<tr>
<td>Heat (37°C for 24 h)</td>
<td>5</td>
</tr>
<tr>
<td>6 M guanidine-hydrochloride</td>
<td>5</td>
</tr>
<tr>
<td>Chloramine T</td>
<td>1</td>
</tr>
<tr>
<td>N-ethylmaleimide</td>
<td>2</td>
</tr>
</tbody>
</table>

* Enzyme preparations were standardized prior to treatment so that 10 µl contained about 1 ng of purified polymerase or 250 ng of disrupted virus. This amount of enzyme directed the incorporation of 25 pmol of [3H]TMP into acid-precipitable material in 1 h at 37°C in the presence of poly(A)-oligo(dT)$_{12}$-$_{18}$, as described in the text. After treatment, remaining enzyme activity was determined.

* Immunological activity was defined as the amount of viral protein or purified polymerase needed to displace 50% of the labeled probe in competition immunoassays.

* Enzyme was denatured by treatment with 6 M guanidine-hydrochloride, pH 6.5, and 10 mM DTT for 72 h at 25°C. The guanidine-hydrochloride was removed by dialysis prior to measurement of remaining enzymatic and immunological activity.

* Enzyme was oxidized by treatment with 100 µg of chloramine T for 1 min at 25°C, followed by metabisulfite reduction.

* Enzyme was exposed to 30 mM N-ethylmaleimide for 15 min at 4°C. Excess reagent was then destroyed by the addition of 200 mM DTT.

**DISCUSSION**

In the present study, radioimmunological techniques have been applied to the identification and quantitation of the reverse transcriptases of mammalian type C viruses. By a combination of ion exchange and affinity chromatography, it was possible to achieve more than a 170-fold purification of the Rauscher MuLV polymerase; the resulting purified protein migrated as a single polypeptide of 80,000 daltons by electrophoretic and gel filtration techniques. This molecular weight is consistent with that reported for the functional enzyme in its native form (7, 20, 21, 46).

Iodinated Rauscher viral reverse transcriptase was shown to be specifically precipitated by antisera directed against mammalian viral polymerases but not by high-titered antisera against other type C viral proteins. These findings argue that the enzyme lacks antigenic cross-reactivity with structural proteins, including gp70, p30, p15, p12, and p10. The virus-coded nature of the polymerase has previously been demonstrated by the isolation of temperature-sensitive mutants of avian and mammalian type C viruses with thermolabile reverse transcriptases (19, 43, 45). In the present studies, competition radioimmunoassays that utilize the iodinated enzyme of Rauscher MuLV demonstrated that reverse transcriptase of the same virus grown in cells of different species remained immunologically unaltered. These results provide independent evidence of the virus-specific nature of the type C viral reverse transcriptase.

Comparison of the immunological reactivities of purified enzyme and disrupted whole virus in an immunoassay for the Rauscher viral polymerase indicated the antigenic equivalent of around 40 enzyme molecules per virion, a value similar to that obtained by an analogous approach for avian myeloblastosis virus (25). These findings are based on the assumption that the immunoreactivity of the viral enzyme is unaltered during purification. The number of molecules per virion of several viral structural proteins is considerably higher (24). Thus, if virus-coded proteins were synthesized in the form of a single large precursor polypeptide, as has been suggested (23), regulation would have to be exerted on the amounts of the final cleavage products actually assembled into the virion. Alternatively, regulation may occur at a transcriptional or translational level.

The Rauscher MuLV reverse transcriptase was shown to possess a wide range of antigenic determinants by analysis in appropriate competition immunoassays. In an assay that uses limiting antiserum directed against the homol-
ogous enzyme, only the polymerase of Rauscher MuLV competed fully. Thus, the specificity of this assay was sufficient to discriminate the Rauscher viral enzyme even from closely related enzymes of other mouse type C viruses. It was not possible to achieve this degree of specificity by antisemum inhibition of the functional activities of Rauscher MuLV polymerase as opposed to other mouse viral enzymes. These findings indicate that the competition immunoassay is more specific for viral reverse transcriptase identification than available immunological methods involving direct polymerase inhibition.

By polymerase inhibition analysis, immunological cross-reactivity has been demonstrated among reverse transcriptases of several mammalian type C virus groups (27, 33). In the present studies, a heterologous competition immunoassay, using antisemum against woolly monkey virus reverse transcriptase to precipitate the Rauscher viral enzyme, demonstrated broadly shared antigenic determinants among the reverse transcriptases of all mammalian type C viruses tested. The antigenic determinants detected by the heterologous immunoassay are more widely shared than the determinants detected by direct polymerase inhibition (33).

Reverse transcriptase has been a major target of investigation as a marker of type C virus expression, largely because its DNA polymerase activity can be sensitively detected by assays using synthetic templates (34). However, since certain cellular DNA polymerases (6, 14), as well as non-type C viral polymerases (26, 32, 36), can also utilize these templates, detection of enzyme activity alone is insufficient to invoke the presence of a type C virus. Further, the lack of RNA-directed DNA polymerase activity does not exclude the presence of these viruses, since enzymological assays cannot detect nonfunctional reverse transcriptase. The present studies demonstrate that radioimmunoassays can readily detect mammalian type C viral reverse transcriptase, independent of its functional state. Moreover, even the broadly reactive heterologous competition immunoassay was still sufficiently specific to exclude from detection reverse transcriptases other than those of mammalian type C viruses. These findings indicate that immunoassays for mammalian type C viral reverse transcriptase should be useful in studying viral enzyme expression in tissues of species known to possess genetically transmitted type C viruses, as well as in searching for viral reverse transcriptase in species from which endogenous viruses have yet to be isolated.

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