Simultaneous Purification of Murine Mammary Tumor Virus Structural Proteins: Analysis of Antigenic Reactivities of Native gp34 by Radioimmunocompetition Assays

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All the structural proteins (gp47, gp34, p27, p23, p16, and p12) of the murine mammary tumor virus (MuMTV) were simultaneously purified utilizing alkyl-agarose chromatography as the initial fractionation step. Least-hydrophobic MuMTV polypeptides (p23, p16) and the slightly hydrophobic p27 were separated from moderately hydrophobic proteins gp47 and p12 by passage through octyl-imino (C_8)-agarose; the gp47 and p12 could be removed from the matrix by elution with ethylene glycol, whereas the most hydrophobic MuMTV protein, gp34, was eluted using nonionic detergent together with ethylene glycol. Subsequent purification steps involved ion-exchange or gel filtration chromatography. The resulting protein preparations appeared near-homogeneous on analysis by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Recoveries of MuMTV proteins, based on their approximate individual contribution to total virus protein, ranged from about 20% for gp47 to greater than 100% for the minor structural component p23, the major phosphoprotein of MuMTV. Antiserum against purified C3H MuMTV gp34, together with purified, radioiodinated gp34, was used to develop a radioimmunoassay which showed that from 13 to 14% of total MuMTV protein by weight is gp34. Using this assay system, the group-specific antigenic reactivity of gp34 was also demonstrated. When solubilized preparations of C3H, RIII, and GR MuMTV's were used as competing antigens in gp34 radioimmunoassays with anti-C3H MuMTV serum, both group- and type-specific differences in antigenic reactivity were found.

The type B murine mammary tumor virus (MuMTV) contains approximately six structural polypeptides, including two glycoproteins (gp34 and gp47) and the nonglycosylated proteins p27, p23, p16, and p12 (5, 13, 21-23, 26, 30). The glycoproteins gp47 and gp34 appear to serve as the extramembrane projections and the intramembrane component, respectively, based on structural dissection in the virions (21, 30) as well as studies on the individual properties of the glycoproteins (12, 22). The nonglycosylated proteins have previously been assigned to the viral core location (12, 22), but recent evidence (4) suggests that p12 may function as a membrane-associated or matrix protein. MuMTV is unique among murine oncornaviruses in containing two glycoproteins and two phosphoproteins. The major phosphoprotein of MuMTV was recently shown to be the minor structural polypeptide p23, and, in addition, a subpopulation of the major core protein (p27) appeared to be phosphorylated (23).

Purification of MuMTV structural polypeptides has been accomplished using a variety of

classical biochemical procedures (1, 6, 15-17, 21, 25, 27, 29). However, the technique of gel filtration in the presence of guanidine hydrochloride, which has proven effective for the purification of type C viral proteins (16), appears to irreversibly denature and inactivate the antigenicity of some MuMTV proteins (16, 17). No single non-denaturing procedure has yet been described by which all of the structural proteins of MuMTV may be purified simultaneously. Additionally, classical purification procedures pose a problem for purification of the extremely hydrophobic membrane-associated peptide, gp34, which has only been purified in the presence of the denaturing detergent, sodium dodecyl sulfate (SDS) (6).

Immunological characterization of MuMTV polypeptides has been reported for gp47 (25, 26), p27 (27), and p16 (1). The major glycoprotein, gp47, was the first soluble MuMTV antigen shown to contain group specificity (17, 21), and subsequent studies have shown that antisera prepared against gp47, p27, and p14 do not cross-react with any other than their homologous

MuMTV protein and that they all contain group-specific antigenic determinants (1, 15, 24, 25, 27). Biological distinctions in the tumorigenicity and hormone dependence of MuMTV's from different mouse strains (14) have prompted a search for the presence of type-specific antigenic determinants on MuMTV structural components. Several laboratories (1, 10, 15) have reported that the use of radioimmunoassay procedures served to identify only group-specific reactivities on the various antigens tested. Recent reports have indicated the presence of both group- and type-specific antigenic determinants on gp47 (24, 25) and p27 (27). Immunochemical characterization of other MuMTV proteins, including gp34, has not been reported.

We have recently reported the use of alkyl-agarose derivatives in determining the relative hydrophobicity (potential for hydrophobic interaction) of both type B and type C murine oncornavirus structural proteins (12) and reverse transcriptases (11). In this paper, we report the development of a protocol for the purification of all six MuMTV structural proteins from a single batch of C3H mouse tissue culture cell-derived virus using hydrophobic chromatography as the initial step in the purification, and without the use of protein-denaturing reagents. Purified gp34 was radioiodinated and used in the development of a radioimmunoassay. Competition studies carried out using homologous virus and solubilized preparations of RIII mouse-derived and GR mouse cell-derived virions revealed the presence of both group- and type-specific antigenic determinants on the gp34 polypeptide.

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MATERIALS AND METHODS

Viruses. The MuMTV that was used for all protein purification was from the Mm5mt mouse mammary tumor virus-producing cell line (7), and was obtained as double isopycally banded, concentrated preparations from the Virus Cancer Program. The GR mouse MuMTV strain was purified from supernatant medium of the GR-3A cell line grown as previously described (19). The RIII MuMTV was obtained from RIII mouse milk and purified as previously described (21). Avian myeloblastosis virus, Rauscher murine leukemia virus, and Mason-Pfizer monkey virus were all obtained as double isopycally banded and concentrated preparations through the Virus Cancer Program.

Reagents. Alkyl-agarose matrices were purchased from Miles Laboratories, Inc. Nonionic detergent P-40 (NP-40) was from Particle Data Co. 125 I for protein iodination was purchased from New England Nuclear

Corp. Sephadex G-75 was obtained from Pharmacia, and phosphocellulose came from Whatman, Inc.

Antisera. Anti-C3H MuMTV serum prepared against whole, solubilized virions was kindly provided by R. Cardiff (serum no. 1) and J. Schlom (serum no. 2). Anti-RIII MuMTV serum was prepared against whole, detergent-solubilized virus as previously described. All of the anti-MuMTV sera used in this study were shown to precipitate both of the MuMTV glycoproteins. Antiserum prepared against C3H-derived MuMTV gp34 purified using sodium dodecyl sulfate (SDS) was obtained through the courtesy of R. Cardiff, and was also prepared in our own laboratory with the participation of J. Racevskis.

Radioiodination of protein. MuMTV gp34 or solubilized virus (2 to 4 μ g of protein), purified using the protocol described herein, was radioiodinated using Iodogen (1,3,4,6-tetrachloro-3 α ,6 α -diphenylglycouril) obtained from Pierce Chemicals, as previously described (13).

Hydrophobic chromatography of MuMTV. Virus disruption, in buffer containing 1% (vol/vol) NP-40, 0.5% (wt/vol) sodium deoxycholate, 10 mM dithiothreitol, 0.4 M KCl, 10% (vol/vol) glycerol, and 0.05 M Tris-hydrochloride (pH 7.8), was carried out as previously described (12). Columns of 2- to 3-ml bed volume containing octylimino (C_8)-agarose and decylimino (C_{10})-agarose were prepared and equilibrated with wash buffer containing 0.05 M Tris-hydrochloride (pH 7.9), 1 mM dithiothreitol, 10% (vol/vol) glycerol, and 1 M KCl at 4°C. The protein concentration of the final disrupted virion solution was kept to below 10 mg/ml, and to avoid overloading no more than 5 mg of protein was applied to any one column of alkyl-agarose matrix. After collection of column flow-through fractions, proteins were eluted with wash buffer containing 8.5 M ethylene glycol, and the most tightly bound MuMTV structural proteins were finally eluted from the columns with wash buffer containing both 8.5 M ethylene glycol and 1% (vol/vol) NP-40. Detailed hydrophobic chromatography procedures for the fractionation of oncornaviral proteins are given in references 12 and 13.

Ion exchange and gel filtration chromatography. A column of phosphocellulose (0.5 by 8 cm) was prepared and equilibrated with buffer containing 0.05 M Tris-hydrochloride (pH 7.8), 1 mM dithiothreitol, 10% (vol/vol) glycerol, and 0.05 M NaCl. The flow rate of the column was maintained at 12 ml/h with the use of a Pharmacia MP-3 peristaltic pump. Molecular sieve chromatography was carried out using Sephadex G-75, which was swelled to final volume in water and equilibrated with buffer containing 50 mM Tris-hydrochloride (pH 7.8), 1 mM dithiothreitol, 0.1% (vol/vol) NP-40, 10% (vol/vol) glycerol, and 0.1 M NaCl. Descending chromatography was performed with a pressure head of 25 cm. The dimensions of the column used were 1.6 by 92 cm.

SDS-PAGE. Polyacrylamide gel electrophoresis (PAGE) was carried out in the presence of SDS and the Laemmli buffer system in 20-cm-long, 5 to 17% (wt/vol) polyacrylamide exponential gradient slabs as previously described (18) or in cylindrical gels as previously described (22). If necessary, proteins were concentrated before SDS-PAGE by either lyophilization

or precipitation with trichloroacetic acid, as previously described (12).

Radioimmunoassays. Normal rabbit serum and goat anti-rabbit serum were purchased from Poconos Rabbit Farms. After radioiodination of MuMTV gp34, free iodine was removed by chromatography on Sephadex G-25, and the material eluting at the void volume was used immediately for immunoassay or stored at 0°C. Titration of the immunoprecipitation of ^{125}I -gp34 was carried out in a volume of 500 μl using serial dilutions of antiserum in a buffer containing 1% (vol/vol) normal rabbit serum, phosphate-buffered saline, 0.1% (vol/vol) Triton X-100, and a fixed amount of labeled protein (12,000 to 20,000 cpm). Incubation was carried out for 2 h at 37°C and overnight at 4°C. The next morning, 50 μl of goat anti-rabbit serum was added to each reaction, mixed, and incubated for 1 h at 37°C and then 3 h at 4°C. Precipitated protein was pelleted by centrifugation for 5 min in a Beckman microfuge, and the resulting pellet was washed once with phosphate-buffered saline solution. ^{125}I label in the precipitate was counted directly in a gamma spectrometer. The concentration of the various antisera necessary to precipitate 50% of maximally precipitable label was determined in this manner and, unless otherwise stated, was routinely used for radioimmunoassay. In competition radioimmunoassays, serial dilu-

tions of unlabeled antigens, as detergent-disrupted virus or unlabeled C3H MuMTV gp34, were used as described above, both with a fixed concentration of antiserum. Virions were detergent disrupted, in a total volume of 100 to 200 μl and at a protein concentration of 1 to 1.8 mg/ml, by the addition of NP-40 and sodium deoxycholate to final concentrations of 0.5% (vol/vol) and 0.1% (wt/vol), and the mixture was incubated for 30 min at 37°C. Protein concentration was determined using the Bio-Rad protein assay (3).

RESULTS

Purification of MuMTV structural proteins. A flow chart of the protocols used to obtain the simultaneous purification of all six MuMTV structural proteins from C3H MuMTV is shown in Fig. 1. We have previously used (C_{10})-agarose for the purification of the least-hydrophobic proteins p23 and p16 from MuMTV, in a procedure which can also be used to isolate R-MuLV pp12 and p10 (13). To extend the purification procedure using hydrophobic chromatography to all of the MuMTV proteins, (C_8)-agarose was used as the initial column step because (i) under the conditions used, p27, p23,

FIG. 1. Flow chart showing the protocols for the simultaneous purification of MuMTV proteins from a single preparation of purified virions. For details of purification refer to the text. Column matrices used in purification are indicated with rectangles. The buffer constituents used for column elution or development are shown to the left side of each appropriate arrow, and the protein(s) which represents the major constituents of each column fraction is shown in parentheses. Circled polypeptide designations represent the final product of the purification.

and p16 are all obtained in the initial column wash (flow-through) fractions (12), and (ii) incomplete recovery of gp34 was observed in studies with (C₁₀)-agarose (12). The peak flow-through fractions from the (C₈)-agarose column were immediately pooled and passed over a (C₁₀)-agarose column, which retained only p27. The p23 and p16 in the (C₁₀)-agarose column wash fractions were initially separated by gel filtration, and complete purification of p16 was achieved by chromatography on phosphocellulose carried out in buffer containing 0.3 M KCl. The basic p16 protein binds to phosphocellulose at 0.3 M KCl and then can be eluted in a single step with 1 M KCl. The major internal MuMTV protein, p27, is eluted from (C₁₀)-agarose with ethylene glycol-containing buffer and purified by a final gel filtration step. It is important to note, for all of the procedures involving hydrophobic chromatography, that this technique utilizes 1 M salt in all wash buffers to minimize the possibility of ionic interactions. Before storage or subsequent chromatography on ion-exchange resins or Sephadex, all fractions were dialyzed against low-ionic-strength buffer and lyophilized as previously described for murine oncornaviral phosphoproteins (13).

Of the MuMTV proteins that remained bound to the (C₈)-agarose column, those that are moderately hydrophobic (gp47, p12) were eluted with ethylene glycol-containing elution buffer (12). After dialysis to remove ethylene glycol and salt (13), the ethylene glycol eluate fraction was concentrated by lyophilization and applied to a phosphocellulose column equilibrated as described in Materials and Methods. The major MuMTV glycoprotein, gp47, was obtained in the phosphocellulose column wash fractions, and the p12 fraction was eluted with buffer containing 0.3 M NaCl. Final purification of both polypeptides was achieved by chromatography through Sephadex G-75 as described in Materials and Methods.

The strongly hydrophobic MuMTV gp34 remained bound to (C₈)-agarose during elution of moderately hydrophobic proteins with 8.5 M ethylene glycol (12). The gp34 was removed from the column by application of buffer containing both ethylene glycol and detergent (see Materials and Methods). A quantity of gp47 was also removed with the gp34, but could be subsequently separated (after dialysis and lyophilization) by gel filtration in the presence of non-ionic detergent. Under these conditions the strongly hydrophobic gp34 was obtained in the void volume of the Sephadex G-75 column (data not shown). The individual preparations of purified MuMTV structural proteins obtained as outlined in Fig. 1 were subjected to SDS-PAGE

analysis as described in Materials and Methods, and the Coomassie blue-stained gel is shown in Fig. 2. From the appearance of the gel the structural protein preparations were nearly homogeneous, indicating that hydrophobic chromatography is efficacious for the purification of all MuMTV structural proteins, and that the extreme hydrophobicity of gp34, which creates a problem when other fractionation methods are attempted, can actually be exploited as an aid to its purification.

Recovery of purified MuMTV structural proteins. To determine the efficiency of purification and the recoveries of the purified polypeptides shown in Fig. 2, the percent contribution of each protein to the total amount of protein present in the virus should be known. The percentage of amino acid label or the intensity of Coomassie blue staining associated with protein bands separated by SDS-PAGE analysis has been previously reported as useful in estimating the fraction of virion protein represented by each polypeptide for MuMTV (5, 22, 26, 30). For the major internal core protein, p27, estimates obtained in this manner were found to be in good agreement with values obtained by the more reliable method of competition radioimmunoassay (13). For the other five MuMTV structural proteins, however, radioimmunoassays either have not been developed or were not

FIG. 2. Purified MuMTV structural protein preparations. SDS-PAGE in the slab gel was carried out as described in the text. (a) 60 μ g of C3H MuMTV protein used as marker for the other lanes; (b) 8 μ g of MuMTV p12; (c) 6 μ g of MuMTV p16; (d) 10 μ g of MuMTV p23; (e) 9 μ g of MuMTV p27; (f) 12 μ g of MuMTV gp34; (g) 13 μ g of MuMTV gp47. Proteins were visualized by staining with Coomassie blue.

used to calculate the percentage of individual protein in isolated virions. We have therefore used the values obtained from SDS-PAGE, which admittedly may be subject to variations due to the methods used, to approximate the percent recovery of virion polypeptides purified using hydrophobic chromatography (Table 1). The best recoveries were observed for the low-molecular-weight MuMTV proteins, with recovery of p23 equaling or exceeding 100% in some preparations, as previously reported (13). Due to the addition of a gel filtration step to remove a contaminating 18,000-dalton protein of uncertain origin, recovery of p27 was less with this procedure than in an earlier report (13), although recoveries in excess of 50% were routinely observed. Recovery of the moderately (p12 and gp47) and strongly (gp34) hydrophobic MuMTV proteins ranged from 20 to 50% (Table 1). This lower recovery relative to the other MuMTV structural proteins may reflect the fact that these proteins bind to and must be eluted from alkyl-agarose columns, with subsequent ion-exchange and/or gel filtration chromatography, and the use of several steps decreases recovery of the viral proteins.

Development of radioimmunoassay for MuMTV gp34. We have previously shown (13) that purification of p27 using alkyl-agarose de-

rivatives yielded preparations which did not have altered antigenic properties, as determined by their ability to compete with p27 in whole, detergent-disrupted, radioiodinated MuMTV preparations. We therefore attempted to develop a radioimmunocompetition assay that would allow the precise quantitation of gp34 within MuMTV virions.

MuMTV gp34 purified as described above was radioiodinated in an Iodogen-mediated reaction as described in Materials and Methods. After removal of the majority of free iodine by gel filtration, the radioiodinated protein was analyzed by SDS-PAGE in cylindrical gels. The ^{125}I -labeled gp34 migrated as a single peak, consistent with the purity of the gp34 preparation (Fig. 2), and indicating that little or no breakdown of the protein had occurred during the iodination process (data not shown). Radioimmunoprecipitation of ^{125}I -gp34 was carried out as described in Materials and Methods, and all anti-MuMTV and anti-gp34 sera used in this study produced a sigmoid radioimmunoprecipitation titration curve similar or identical to those previously described (24, 25, 27). From 60 to 90% of the labeled gp34 could be precipitated with appropriate antisera. In the absence of specific antiserum, however, <2% of input radioactivity was found in the pellet after incubation with goat anti-rabbit serum (data not shown). Radioimmunoassays for gp34 using antiserum to SDS-purified C3H gp34 and purified, unlabeled C3H MuMTV gp34 or detergent-disrupted virions of C3H MuMTV, RIII, MuMTV, and GR MuMTV as competing antigens are shown in Fig. 3. This study indicated that gp34 comprises approximately 13 to 14% of the total MuMTV protein, a value in good agreement with the more conservative approximations reached by the analysis of stained gels after SDS-PAGE (22, 30). The anti-C3H gp34 serum used in this study did not react with solubilized preparations of avian myeloblastosis virus, Mason-Pfizer monkey virus, or Rauscher murine leukemia virus, even at high virus protein concentrations. This result indicates the presence of strong group-specific antigenic determinants on gp34.

Analysis of type-specific antigenic determinants on gp34. Previous reports have demonstrated that, when using antiserum directed against the purified MuMTV gp47 (24, 25) and p27 (27) in radioimmunoassays, type-specific as well as group-specific antigenic determinants may be found on these structural proteins. The fact that, in radioimmunoassays utilizing antisera against SDS-purified C3H MuMTV gp34, all three strains of MuMTV tested showed identical immunocompetition

TABLE 1. Recovery of MuMTV proteins purified using hydrophobic chromatography

Poly-peptide	Protein in final prepn		Individual polypeptide/total protein in virus ^c (%)	Approx. % recovery of individual protein ^d
	mg ^a	Per-cent ^b		
gp47	0.7	7	26-31	22-27
gp34	0.6	6	16-21	29-37
p27	0.9	9	16-18	50-56
p23	0.24	2.4	1-3	>100
p16	0.7	7	7-9	80-90
p12	0.3	3	6-8	37-50

^a Purified MuMTV preparations (10 mg of protein) were used in a representative purification. The final purified products obtained were analogous to the individual polypeptide preparations shown in Fig. 2.

^b Variations of 10 to 20% were observed in different experiments.

^c Values for the approximate percentage of total virion protein represented by each polypeptide were obtained from the percentage of total radioactivity in each protein peak after SDS-PAGE using virions labeled in vivo with amino acids (30) and from the relative intensity of Coomassie blue stain associated with each protein band in similarly analyzed virions (22).

^d Expressed as the ratio of percent input protein to the percent contribution of the individual polypeptide to total viral protein.

curves as well as ability to completely compete with C3H gp34 (Fig. 3) suggested a possible absence of type-specific determinants. However, the possibility existed that purification in the presence of the denaturing agent SDS may have altered the conformation of the protein such

FIG. 3. Competition radioimmunoassay for C3H MuMTV gp 34 using antiserum prepared against SDS-purified C3H gp34 and ^{125}I -gp34 purified as described in Fig. 1. Antiserum was used at a final dilution of 1:15,000, which was sufficient to precipitate 50% of maximally precipitable label. Detergent-disrupted, solubilized avian myeloblastosis virus ($\times$), Rauscher murine leukemia virus ($\blacktriangledown$), and Mason-Pfizer monkey virus ($\square$) were used as competing antigens to detect group-specific antigenic reactivity. Solubilized C3H ($\bullet$), GR ($\circ$), and RIII ($\triangle$) MuMTV were also used as competing antigens in addition to purified C3H gp34 ($\blacksquare$) to quantitate the percent contribution of that polypeptide to total MuMTV protein. Radioimmunocompetition assays and virus solubilization were carried out as described in the text.

that, when antisera was raised against it, a population of antibodies against type-specific determinants may have been absent. Radioimmunoassays utilizing antisera against whole MuMTV and purified iodinated gp47 have been shown effective for the identification of type-specific reactivity (24, 25). Therefore, several preparations of anti-C3H MuMTV serum were obtained (see Materials and Methods) and used in the radioimmunoassay analysis. Because previous studies indicated that low dilutions of antiserum provided increased sensitivity to type-specific differences (24), we used two dilutions of antiserum, one of which was sufficient to precipitate 50% of the maximally precipitable quantity of ^{125}I -gp34 whereas the other was 10- to 15-fold less diluted. Anti-C3H MuMTV serum no. 2 used in this study (Fig. 4) showed strong group specificity on gp34 at low serum dilution (Fig. 4A). With this specific anti-C3H MuMTV serum at this concentration, differences were observed both in slopes of the immunoprecipitation competition curves among MuMTV of C3H, GR, and RIII mouse origins and in the extent of final competition with labeled antigen at high concentrations of competing antigens. Although the homologous C3H virus was able to completely compete with labeled antigen for antibody, detergent-disrupted GR and RIII MuMTV only competed to a maximum of 60%. When the same anti-C3H MuMTV antiserum was used at a final dilution of 1:180,000 (the concentration capable of precipitating 50% of maximally precipitable labeled antigen), the slopes of competing antigens from all three types of MuMTV appeared quite similar. However, it was evident that only the homologous C3H MuMTV provided sufficient antigenic determinants to allow complete competition with the

FIG. 4. Radioimmunocompetition assay for MuMTV gp34 using anti-C3H MuMTV serum no. 2 (see the text) at (A) 1:10,000 and (B) 1:180,000 final dilution (that dilution of serum allowing 50% precipitation of labeled antigen). Competing antigens were as described in the legend to Fig. 3.

labeled gp34. The GR and RIII MuMTV's competed to 85 and 90%, respectively (Fig. 4B), even at 10-fold higher protein concentrations than those shown in the figure. Therefore, type-specific antigenic determinants on the gp34 molecule were observed using anti-C3H MuMTV serum no. 2, and the presence of such determinants could be amplified through the use of low dilutions of antiserum.

Using rabbit serum no. 1 to C3H MuMTV (see Materials and Methods) at low (1:10,000) and high (1:100,000) dilution, the slopes of the immunocompetition curves for C3H, RIII, and GR viruses appeared identical (Fig. 5). The failure of GR MuMTV to compete completely with labeled antigen was the only apparent sign of type-specific antigenic differences, although group-specific reactivity was still apparent (Fig. 5A). Two other anti-C3H MuMTV sera prepared and used in this study at high and low dilution revealed no type-specific immunoreactivity on gp34 for the viruses examined. Therefore, our studies using various rabbit antisera prepared against C3H MuMTV show that the ability to identify type-specific antigenic determinants on MuMTV gp34 is dependent on the specific antiserum used.

DISCUSSION

Hydrophobic chromatography has only recently been applied to the study of oncornavirus proteins (11-13). We have shown that these matrices can be used to determine the potential for hydrophobic interaction (relative hydrophobicity) of structural proteins, and have arbitrarily defined four categories of increasing relative hydrophobicity based on these procedures (12). The fractionation of viral structural proteins observed on these matrices naturally suggested

their use as reagents in protein purification. The lack of binding of both murine type C and type B phosphoproteins to (C₁₀)-agarose has been reported as a rapid and effective method for their purification (13). Modification of this protocol, as described above (Fig. 1), has allowed the simultaneous purification of all the MuMTV proteins, which appear near-homogeneous upon SDS-PAGE analysis, as determined by Coomassie blue staining (Fig. 2). Traces of contaminating low-molecular-weight proteins of uncertain origin are, however, sometimes seen in p23 preparations (13). The fact that gp34 aggregates in aqueous solution prompted other investigators to resort to the use of SDS in its purification (6). Methods such as lectin-agarose chromatography (20, 29) as well as ion-exchange chromatography (6), which have proven successful in the purification of gp47, are not useful for gp34 isolation. Indeed, gp47 and gp34 copurify through a variety of fractionation steps (29) and do so even to a degree during hydrophobic chromatography (12). Therefore, in addition to providing a procedure for the simultaneous isolation of all six MuMTV structural proteins in good yields (Table 1), the protocol shown in Fig. 1 represents the first method for purifying gp34 without the use of denaturing reagents, through the exploitation of its extremely hydrophobic nature. It should be mentioned, however, that the MuMTV DNA polymerase is not a likely candidate for future purification on alkyl-agarose matrices, since, in contrast to structural proteins, it binds to both (C₈)- and (C₁₀)-agarose with such avidity that neither ethylene glycol-containing buffer nor the same buffer also containing detergents is able to elute active enzyme (11).

By preparing a radioimmunoassay with purified gp34, we were able to quantitate

FIG. 5. Radioimmunoassay for C3H MuMTV gp34 using anti-C3H MuMTV serum no. 1 (see the text) at dilutions of (A) 1:10,000 and (B) 1:100,000 (that dilution of antiserum allowing 50% precipitation of labeled antigen). Symbols for competing antigens are identical to those defined in the legend to Fig. 3.

tate the amount of gp34 present in mature virus. We estimated that gp34 accounts for approximately 13 to 14% of the total protein in purified virions. The absence of deoxycholate, or an increase in virus protein concentration in the solubilization reaction mixture above 1.5 mg/ml, resulted in underestimation of the percentage of gp34 in the virion (data not shown). This result may be due to the tenacious association of gp34 with the virus membrane, to its lipophilic properties, to its tendency to aggregate in aqueous solution (which perhaps results in the masking of certain antigenic sites), or to all of these. The fact that lipophilic proteins bind large quantities of detergents (9) suggests that the weight ratio of protein to detergent may be a critical factor in the solubilization of certain virus proteins.

Although several reports using differing immunological techniques have shown the presence of group-specific antigenic determinants associated with MuMTV (1, 15, 16, 21), identification of type-specific determinants on purified MuMTV proteins have only been reported for gp47 (24, 25) and p27 (27). Blair (2) was the first to report the possible presence of type-specific differences among MuMTV's, using whole virus preparations in immunodiffusion analysis. We have been able to demonstrate the apparent existence of both group- and type-specific antigenic reactivities on the gp34 polypeptide (Fig. 3 to 5) using detergent-solubilized preparations of the homologous (C3H) MuMTV and RIII and GR mouse cell-derived MuMTV's as competing antigens. Of three different anti-C3H MuMTV sera tested having similar or identical radioimmunoprecipitation titers for gp34, only one (serum no. 2, Fig. 4) showed distinct type-specific differences in gp34 antigenic reactivity; the RIII and GR viruses were unable to completely compete with C3H gp34 for the available antibody population. Teramoto and Schlom (28) have obtained similar results with antiserum no. 2. Type-specific antigenic determinants on MuMTV gp34 also appear to be most demonstrable at low antiserum dilution (Fig. 4A). Although only one of the sera tested showed clear type-specific differences, all anti-C3H MuMTV sera used in this study demonstrated group-specific reactivities on MuMTV gp34. The finding of type-specific antigenic reactivities on a purified viral protein with one antiserum may therefore not necessarily mean that identical results will be achieved with other antiviral sera, even if they show comparable titers in radioimmunoprecipitation assays.

Tryptic peptide fingerprinting analysis of radioiodinated viral proteins has recently been used to analyze possible strain-specific differ-

ences in the gp47, gp34, and p27 components of RIII, C3H, and GR MuMTV (8). Differences in the peptide maps of gp47 and p27 were found consistent with previous descriptions of type specificity (24, 25, 27). However, significant differences in the gp34 peptide maps from all three virus strains were not detected (8). These results are in contrast to the demonstration of type-specific determinants by at least one anti-MuMTV serum in our studies (Fig. 4). It is possible that this antiserum contains a unique population of antibodies to specific and perhaps minor changes in glycosylation of the C3H gp34 as compared with other gp34 molecules of the RIII and GR MuMTV strains. These changes would not be detectable in peptide mapping. Alternatively, type-specific differences may reside in peptides lacking tyrosine residues, which would be undetected by the procedure used for the analysis of the peptide maps of MuMTV proteins (8). We are currently preparing antisera against gp34 purified by the above procedure (Fig. 1 and 2) to determine whether antiserum prepared against the native form of gp34 can be used to detect type-specific reactivities on this protein.

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