JOURNAL OF VIROLOGY, July 1975, p. 53-61
Vol. 16, No. 1

Printed in U.S.A.

Chromatographic Separation and Antigenic Analysis of Proteins of the Oncornaviruses

V. Identification of a New Murine Viral Protein, p15(E)

HISAMI IKEDA,* WILLIAM HARDY, JR., ELLEN TRESS, AND ERWIN FLEISSNER

Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Received for publication 13 January 1975

Profiling of murine leukemia virus (MuLV) proteins by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) has revealed a low-molecular-weight protein which does not appear in the corresponding region of viral protein profiles obtained by gel filtration in 6 M guanidine hydrochloride. This protein species, termed p15(E), is easily demonstrable in MuLV isolates for which the viral p15 and p12 proteins have almost identical electrophoretic mobilities; this leaves a protein slightly larger than these two in the PAGE system unaccounted for in the gel filtration system. However, antiserum against the void volume fraction of the gel filtration eluate precipitated the p15(E) component from solubilized, radiolabeled virions, as shown by SDS-PAGE analysis of such immunoprecipitates. Comparative radioprecipitation analyses of this type revealed that for various MuLV isolates p15(E) was distinguishable from p15 in terms of serological reactivities, relative mobilities in gel electrophoresis, and relative efficiencies of labeling with individual amino acids. Thus it appears that, as is the case for avian oncornaviruses, MuLVs contain seven major structural proteins.

In the past several years a number of complementary methods have been used to study structural proteins of oncornaviruses (1, 2, 19). These include polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS), gel filtration in 6 M guanidine hydrochloride (GuHCl), isoelectric focusing in sucrose or in urea of virus disrupted with nonionic detergent, and chromatography of proteins from disrupted virus on phosphocellulose. The latter three methods have permitted the recovery of native virion antigens. From the combined application of these methods has emerged the concept that the number of virion structural proteins is small (six or seven), consistent with recent reports concerning the nucleotide sequence complexity of viral nucleic acid (5, 22). There is also evidence that most of these structural proteins are virus coded. Thus it can be demonstrated that viral proteins retain their characteristic serological reactivities when synthesized in host cells of a different species and yield peptide fingerprints peculiar to the particular virus strain of origin when several strains of virus are grown in the same type of host cell (4, 9).

For the murine leukemia viruses (MuLVs) considerable agreement exists on the properties of certain structural proteins, e.g., the major viral core protein, p30, and the major viral envelope glycoprotein, gp70. By contrast, in recent reports concerning the characterization of MuLV proteins with molecular weights estimated at 15,000, one group found a protein that was mainly type specific and presumably internal to the virion (24); the other found a strongly group-specific protein, located on the exterior of the viral envelope (16, 22). In analyzing a number of endogenous MuLV isolates, as well as Gross-MuLV and Friend-, Moloney-, and Rauscher (FMR)-type MuLVs, we have found that, although these show the usual protein pattern in gel filtration, including the protein designated p15, they display an additional protein with a molecular weight near 15,000 by PAGE analysis. In this paper we demonstrate that this new protein, MuLV p15(E), is distinguishable from MuLV p15 by serological and other procedures. The existence of two MuLV proteins in this size range resolves the above mentioned paradox concerning the properties of such proteins and establishes that MuLVs, like their avian counterparts, contain seven major structural polypeptides.

MATERIALS AND METHODS

Cells and viruses. Cell types in which viruses were grown are specified in the appropriate figure legends

and tables. Stocks of mouse cells and viruses, originally from J. W. Hartley and W. P. Rowe of the National Institute of Allergy and Infectious Diseases, were kindly supplied by T. Pincus of this Center. Besides Gross, Moloney, and Rauscher MuLVs (G-, M-, and R-MuLVs), these included the N- and B-tropic BALB/c isolates, WN1802N and WN1802B (previously referred to as BALB/c-S2N and BALB/c-S2B [14]), originally derived from the spleen of a single BALB/c mouse, N- and B-tropic C57BL/6 viruses, also from a single mouse, and N-tropic AKR virus. A B-tropic C3H isolate was obtained from N. Sarkar, Memorial Sloan-Kettering Cancer Center, and N-tropic Friend virus (F-MuLV) from F. Lilly, Albert Einstein College of Medicine, New York. Secondary mouse embryo cell cultures from NIH Swiss mice were infected with G-MuLV at multiplicities of about 2 XC PFU/cell as described (14), and virus was harvested 4 to 5 days later. For other MuLVs, infected cultures of III6A mouse embryo cells (13) were maintained as producer lines, in T-75 plastic flasks (Falcon Plastics), using minimal Eagle medium (6) supplemented with antibiotics and 10% fetal bovine serum (Microbiological Associates). The R-MuLV-producing rat cell line RRTC (18) was maintained in the same medium. Feline lung cells were infected with feline leukemia virus as described (12). Cells for immunofluorescence adsorption studies were trypsinized, pelleted in the presence of fetal bovine serum, disrupted by sonic treatment, and clarified at 3,000 × *g* for 20 min.

MuLVs labeled in tissue culture with ^{3}H- or ^{14}C-labeled amino acid precursors were grown and purified as previously described (18, 19).

Fractionation of viral proteins. PAGE in the presence of SDS (SDS-PAGE) was performed according to published procedures (7, 19), using 16 cm, 10% acrylamide, and 0.33% bisacrylamide gels which were subjected to 4.4 V/cm for 16 h. Gels were fractionated in an automatic gel divider (Gilson Medical Electronics), and the fractions were counted in a scintillation counter, using a triton:toluene (1:2) scintillation mixture.

Isolation of viral proteins by gel filtration in 6 M GuHCl for the experiments shown in Fig. 1 to 3 was performed as previously described, utilizing MuLVs labeled in tissue culture with mixed ^{14}C-labeled amino acids. Isolated viral proteins in the column eluate were dialyzed free of GuHCl and lyophilized prior to use in PAGE (7).

Serological procedures. Antisera were made in New Zealand white rabbits against (RRTC grown) R-MuLV protein fractions isolated by gel filtration in 6 M GuHCl, followed by extensive dialysis (7). The initial immunization in complete Freund adjuvant and two boosters in incomplete Freund adjuvant were administered at intervals of 4 weeks. Approximately 90 μg of viral protein was contained in each injection.

A rabbit antiserum prepared against a 15,000 molecular weight fraction from F-MuLV (22) was kindly supplied by D. Bolognesi, Duke University Medical Center, and a goat antiserum against R-MuLV p15 (24) was a gift from M. Strand and J. T. August, Albert Einstein College of Medicine.

Immunofluorescence and immunofluorescence absorption were carried out according to published procedures (17), utilizing acetone-fixed cells stained with fluorescein-conjugated goat anti-rabbit immunoglobulin G (IgG) (Hyland Labs.).

Immunoprecipitation and electrophoretic analysis of radiolabeled proteins precipitated by specific antisera were adapted from Ihle et al. (15). Radioactively labeled virus, banded by sucrose density gradient centrifugation, was diluted in TNE (0.01 M Tris-hydrochloride [Sigma], pH 7.4, 0.1 M NaCl, 1 mM EDTA) and pelleted for 1 h at 24,000 rpm in a Beckman SW27 rotor. Viral pellets were dissolved in 0.5 M NaCl, 0.02 M Tris-hydrochloride, pH 7.4, and 0.5% Nonidet P-40 (NP-40, Shell Chemicals) and were incubated at 37 C for 10 min. Hyperimmune rabbit serum was added to a final dilution of 1:200 in 2 ml and incubation was carried out at 37 C for 90 min. Goat anti-rabbit IgG (Hyland Labs.) was then added at a 10:1 ratio to the hyperimmune serum (or equivalency, as determined by appropriate titrations) and incubation was continued at 37 C for 30 min and at 0 C for 1 h. Precipitates were collected by centrifugation at 3,000 × *g* for 20 min in an International PRJ centrifuge. The precipitates were washed once in 1 M NaCl, 0.02 M Tris-hydrochloride, pH 7.4, 0.001 M EDTA, and 0.5% NP-40, and once in 0.002 M Tris-hydrochloride, pH 7.4 and 0.5% NP-40 and drained well before solubilization for analysis by SDS-PAGE.

RESULTS

MuLV p15(E) can be visualized by PAGE in SDS but not by gel filtration in GuHCl. In Fig. 1A is shown the profile obtained for proteins of the N-tropic BALB/c isolate, WN-1802N, by gel filtration in 6 M GuHCl, and in Fig. 1B are shown the results of electrophoresing the isolated p15 and p12 proteins with marker virus. The designations used for the viral protein species have been discussed previously (1); the numbers refer to apparent protein molecular weights ($\times 10^{-3}$). The pattern of viral proteins in gel filtration is similar to that reported for F-, M-, R-, and G-MuLVs (4, 11), in showing three well-resolved protein species smaller than p30. The PAGE pattern also shows three clearly resolved peaks of higher mobility (lower molecular weight) than p30. However, one of the PAGE peaks in this lower-molecular-weight range accounts for two of the smaller protein species obtained by gel filtration (p15 and p12) (Fig. 1B). For reasons explained below we have designated as p15(E) the protein species in PAGE which is thus left unaccounted for.

In Fig. 2 are shown the results of PAGE analysis of G-MuLV p15, p12, and p10 isolated by gel filtration. The results are analogous to those for the BALB/c isolate: p15 and p12 coalesce in a single peak in PAGE; p10 behaves

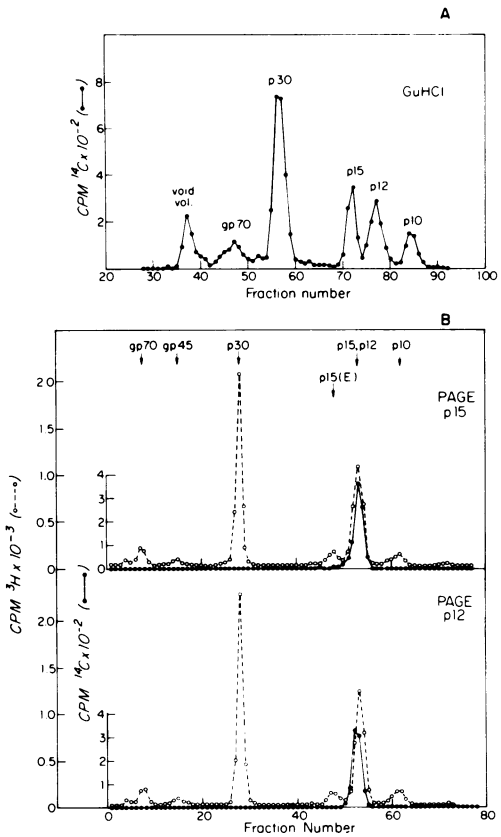

FIG. 1. *(A) Gel filtration in 6 M GuHCl and 0.01 M dithiothreitol of proteins from WN1802N virus labeled in vivo with mixed ^{14}C-labeled amino acids (4, 18). (B) Co-electrophoresis in SDS-containing polyacrylamide gels of ^{3}H-amino acid-labeled WN1802N virus with individual ^{14}C-labeled MN1802N proteins p15 and p12 obtained from the experiment shown in (A). Direction of migration in PAGE is from left to right. Virus was grown in III6A mouse embryo cells.*

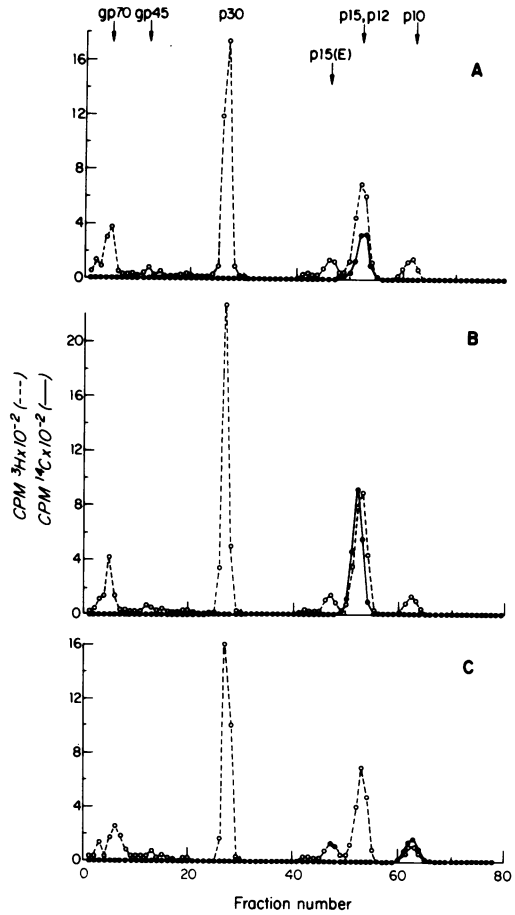

FIG. 2. *SDS-PAGE analysis of ^{14}C-labeled G-MuLV proteins p15 (panel A), p12 (panel B), and p10 (panel C). The experiment was analogous to that depicted in Fig. 1B: the individual proteins obtained by gel filtration in 6 M GuHCl were co-electrophoresed with ^{3}H-labeled G-MuLV proteins as marker. For the p10 preparation, 50 μg of bovine serum albumin was added before dialyzing away GuHCl. G-MuLV was grown in NIH Swiss mouse embryo cells.*

as expected; and the p15(E) species is again clearly discernible. The following viruses display PAGE patterns similar to those for G-MuLV and the N-tropic BALB/c virus: N-tropic AKR and C57BL/6 MuLV isolates and B-tropic BALB/c, C57BL/6, and C3H isolates. In addition, M-MuLV behaves like these endogenous viral isolates in our PAGE system, whereas R-MuLV and F-MuLV do not. Thus R-MuLV p15 and p12 are readily resolvable in PAGE (Fig. 3). (The virus for this experiment was produced in rat cells; similar profiles are obtained for R-MuLV grown in mouse cells.) The protein peak corresponding to the p10 species visualized by gel filtration in GuHCl is not always discernible in multiple PAGE runs on the same virus preparation, probably due to the tendency of this species to aggregate in PAGE (cf. Fig. 3C and reference 9). A protein with the characteristics of the p15(E) protein of G-MuLV and endogenous viral isolates was identifiable in the FMR group of viruses by serological criteria, described subsequently in this report. When G-MuLV and R-MuLV proteins were profiled simultaneously in PAGE (not true for Fig. 2 versus Fig. 3), their p15(E) species had identical mobilities. With regard to the component designated "x" in Fig. 3, this protein is consistently present (in varying amounts) or consistently absent in different preparations of the same virus. This variability, and the kinetics of labeling of component "x" during

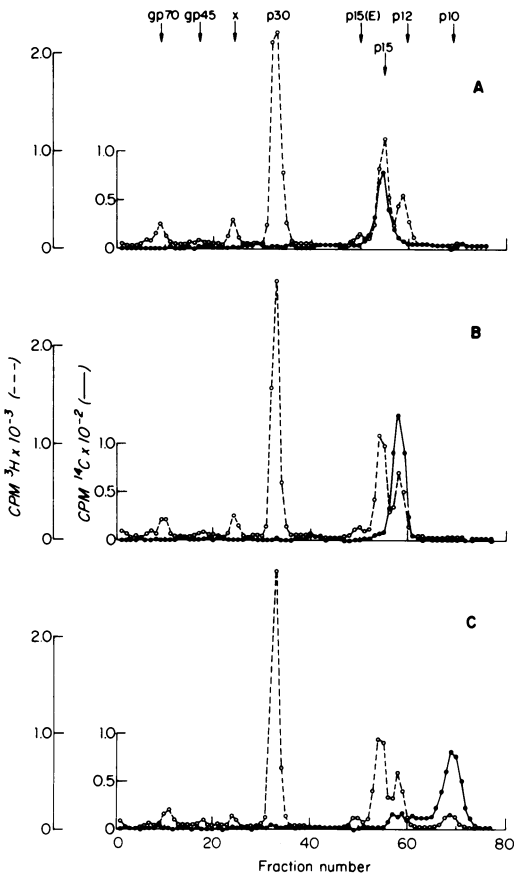

FIG. 3. *SDS-PAGE analysis of R-MuLV proteins p15 (panel A), p12 (panel B), and p10 (panel C). Co-electrophoresis of ^{3}H-amino acid-labeled R-MuLV with individual R-MuLV proteins p15, p12, and p10, isolated from ^{14}C-amino acid-labeled R-MuLV by gel filtration in 6 M GuHCl (cf. Fig. 1). The occasional anomalous behavior of MuLV p10 is illustrated in the figure. Note aggregation of purified p10 in (C) despite addition of carrier protein during dialysis (cf. 9). R-MuLV was produced in the RRTC rat cell line.*

single cycle viral growth (9), lead us to conclude that this polypeptide, with a molecular weight of about 40,000, represents a protein of the actin type, which is a major host cell constituent and occasional contaminant of virus preparations (8); Allan Goldberg, personal communication).

Detection of MuLV p15(E) with antiserum against the void volume fraction of MuLV proteins separated by gel filtration. To establish the viral specificity of p15(E), a serological approach was used. Ihle and associates (15) have recently published a simple method for determining the specificities of various antisera for particular virion proteins of MuLVs. In essence this procedure involves solubilization of radioactively labeled, pelleted virus in a high-salt buffer containing a nonionic detergent (NP-40). Under these conditions antigen-antibody interactions proceed normally. Reaction with a specific serum followed by anti-Ig permits recovery of a precipitate which can then be analyzed by SDS-PAGE to determine the radioactive viral proteins which have been recognized by the specific antiserum. We have verified the selectivity of this procedure in singling out individual proteins in the solubilized virion preparation and have applied the procedure to analysis of hyperimmune sera prepared against purified viral protein fractions.

Antisera were prepared in rabbits against the individual, rechromatographed R-MuLV protein species isolated by GuHCl-gel filtration and renaturation (9). Four sera, against the void volume fraction, gp70, p30, and p15, were initially characterized by immunofluorescence titration on virus-infected tissue culture cells (p12 and p10 were not effective immunogens in the immunization schedule employed). Table 1 shows the immunofluorescence titers obtained for the four reactive antisera with several types of virus-infected and control cells. The results for anti-void volume and anti-p15 sera are of particular interest; the results with anti-p30 and anti-gp70 sera have been alluded to previ-

TABLE 1. *Titers of rabbit antisera against Rauscher proteins in immunofluorescence tests with various cell lines*[a]

Test cells	Anti-void volume[b]	Anti-p15	Anti-p30	Anti-gp70
Mouse cells[c] infected with:				
R-MuLV	640	320	640	320
G-MuLV	640	20	320	10
M-MuLV	640	80	320	20
Control mouse cells	10	<10	<10	<10
Feline cells[d] infected with FeLV	40	<10	80	10
Control feline cells	40	<10	<10	10

[a] The antiserum titer (end point) is expressed as the highest dilution showing bright cytoplasmic fluorescence. The brightness is rapidly lost beyond those dilutions.

[b] The void volume protein fraction obtained by gel filtration of R-MuLV proteins and dialysis free of GuHCl.

[c] NIH Swiss mouse embryo fibroblasts in secondary passage.

[d] Feline lung fibroblasts. FeLV, Feline leukemia virus.

ously (17) and are included here for purposes of comparison. The rabbit antisera to these four protein fractions obtained by gel filtration displayed specific granular cytoplasmic fluorescence with virus-infected cells. Uninfected cells were immunofluorescence negative. Antiserum to the void volume fraction had a similar immunofluorescence titer with R-MuLV, G-MuLV and M-MuLV infected cells; this group-specific activity was comparable in strength to that of antiserum against R-MuLV p30. In contrast, the titer of anti-p15 was higher against cells infected with R-MuLV, compared to cells infected with M-MuLV and particularly with G-MuLV, consistent with major type-specific determinants in this protein, as in the case of R-MuLV gp70 isolated by the gel filtration method (cf. 17). Of the four antisera, only that prepared against gp70 displayed neutralizing activity against R-MuLV in the XC plaque test (T. Pincus, personal communication). Immunofluorescence absorption tests with purified MuLV preparations and sonically treated extracts of MuLV-infected cells, as well as with MuLV protein fractions isolated by gel filtration, confirmed the distinctive and separate specificities of the four antisera.

When the method of Ihle et al. (15) was employed to analyze the anti-void volume and anti-p15 sera, the latter was found to precipitate p15, as expected; however it was surprising to find that the antiserum against the void volume fraction precipitated p15(E), as well as viral glycoproteins. Thus p15(E) apparently eluted in the void volume fraction of the gel filtration eluate, accounting for the absence of this species in the lower-molecular-weight region of the elution profile, as well as in PAGE profiles of peaks from this region (Fig. 1, 2, and 3).

Figure 4 shows the results of a typical antibody precipitation experiment with G-MuLV and anti-void volume serum, a combination designed to detect group-specific antigens. The top panel shows the pattern of total viral proteins (mixed amino acid label) and glycoproteins (glucosamine label) in SDS-PAGE. The bottom panel shows the results obtained by reacting an equal amount of the same labeled virus preparation, solubilized in NP-40, with the anti-void volume serum (diluted 1:200) and anti-Ig, followed by analysis of the washed precipitate in SDS-PAGE. The most prominent labeled protein found in the precipitate was p15(E), which was recovered quantitatively. The anti-void volume serum was actually expected to contain antibody against gp45 (1, 18); however both gp70 and gp45 (the latter better

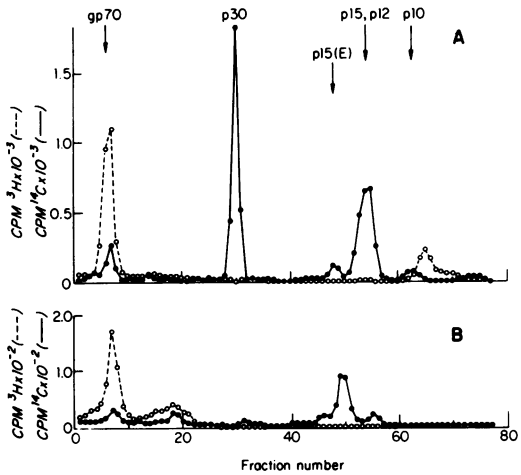

FIG. 4. *PAGE profiles obtained for (A) total G-MuLV proteins labeled with [^{3}H]glucosamine and [^{14}C]-labeled amino acids and (B) proteins immunoprecipitated from an equal amount of the same virus preparation by antiserum prepared against the void volume fraction of R-MuLV subjected to gel filtration in 6 M GuHCl. G-MuLV was grown in NIH Swiss mouse embryo cells.*

visualized than in the protein pattern from whole virus) were brought down, probably due to an NP-40 stable association of these two proteins. A similar association, using R-MuLV as the test virus, was observed with our anti-gp70 serum and with an antiserum against a glycoprotein preparation obtained by another method (anti-R-MuLV gp69/71 serum from J. T. August [17, 23]). As expected, the void volume antiserum identified R-MuLV p15(E) as well as viral glycoproteins. Anti-p30 and anti-p15 sera identified their respective proteins from R-MuLV, confirming the selectivity of the precipitation procedure, as demonstrated also by Ihle et al. (15, 16).

Differential labeling of MuLV p15(E) with single amino acids. The identity of the low-molecular-weight protein brought down by the void volume serum was confirmed by growing virus in the presence of pairs of differentially labeled amino acids and determining the relative incorporation of the two isotopes into viral proteins separated by SDS-PAGE. Results obtained for R-MuLV and G-MuLV using [^{3}H]lysine and [^{35}S]methionine are shown in Table 2. The met/lys ratios for the low-molecular-weight protein precipitated with anti-void volume serum are the same for R-MuLV and G-MuLV and match closely the values for p15(E) in the virus preparations, differing quite significantly from the values obtained for p15, p12, and p10. (The match is not exact for G-MuLV, due to the

influence of the large neighboring peak of lysine-rich, methionine-free p15, on the ratio in the p15(E) position in the PAGE pattern.) Analogous results were obtained by [^{3}H]leucine and [^{14}C]arginine labeling. Thus p15(E) is recognized by void volume serum and is distinct from p15, p12, and p10 by this criterion.

TABLE 2. *Met/lys ratios in MuLV proteins analyzed by PAGE of virus and immunoprecipitates*[a]

Proteins	R-MuLV	G-MuLV
p15(E)	0.17	0.11[b]
p15	0.04	0.00
p12	0.50	
p10	0.00	0.00
ppt'd p15(E)	0.17[c]	0.17[c]

[a] Expressed as ratios of counts per minutes incorporated. Both R-MuLV and G-MuLV were grown in NIH Swiss mouse embryo cells.

[b] The value for this protein peak is lowered since it is not completely separated from the neighboring peak (p15 plus p12), which is very rich in lysine and contains no methionine in the case of G-MuLV.

[c] The major protein species, with a molecular weight of about 15,000, precipitated by the anti-void volume serum.

Presence of p15(E) in other MuLV isolates and serological comparison to p15. A comparison of the reactivities of anti-void volume serum and anti-R-MuLV p15 by PAGE analysis of immunoprecipitates is shown in Fig. 5 for M-MuLV and F-MuLV. The p15(E) protein in both viruses is specificially recognized by the anti-void volume serum, together with proteins having the mobilities of viral glycoproteins (Fig. 5A, D). As indicated in the discussion of Fig. 4, our tentative explanation for precipitation of both viral glycoproteins by this serum (unexpected since gp70 is not detectable in the void volume) is that this is due to a combination of gp70 and gp45 from NP-40-lysed virus in a complex which is stable to NP-40. We have placed the term gp45 in parentheses in this figure because the molecular weight of the smaller glycoprotein component appears to be considerably higher than 45,000 for M-MuLV

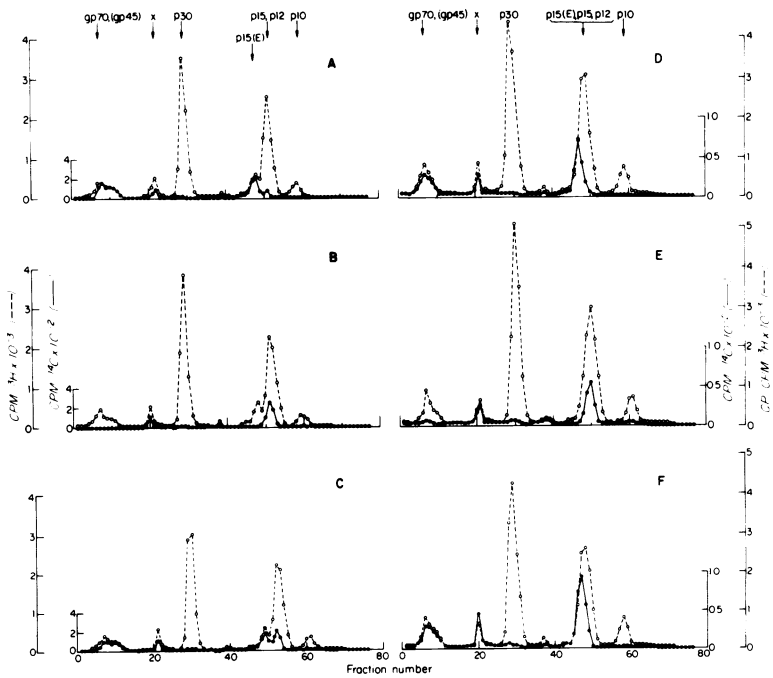

FIG. 5. *PAGE profiles obtained for ^{14}C-amino acid-labeled M-MuLV and F-MuLV proteins, precipitated with specific antisera and co-electrophoresed with ^{3}H-amino acid-labeled total proteins of the corresponding viruses. (A) M-MuLV proteins precipitated by antiserum prepared against the void volume fraction of R-MuLV subjected to gel filtration in 6 M GuHCl. (B) M-MuLV protein precipitated by the antiserum to R-MuLV p15, similarly prepared. (C) M-MuLV proteins precipitated by an antiserum against a 15,000-molecular-weight protein fraction from F-MuLV (23). (D) F-MuLV proteins precipitated by the R-MuLV anti-void volume serum. (E) F-MuLV protein precipitated by the anti-R-MuLV p15 serum. (F)-MuLV proteins precipitated by the same serum used in (C), which recognizes both F-MuLV p15 and p15(E). Viruses were grown in III6A mouse embryo cells.*

and F-MuLV, appearing as a shoulder on the gp70 peak. Owing to the difficulty in obtaining a characteristic, sharp band for this component in SDS-PAGE analysis of most MuLV isolates, estimates for its molecular weight must be regarded as approximate at this time. Although the p15(E) component detected by anti-void volume serum is not well resolved from p15 and p12 in the case of F-MuLV, it has a characteristic mobility lower than that of the other proteins in the region of 12,000 to 15,000 molecular weight in the gel. (F-MuLV p12 has a somewhat higher mobility than F-MuLV p15 in SDS-PAGE [11].) As shown in Fig. 5B and E, for both viruses (which would be expected to share type-specificities with R-MuLV, as members of the FMR group of MuLVs [20]), anti-p15 serum precipitated a single protein species of higher mobility than the principal species recognized by the anti-void volume serum; i.e., MuLV p15(E) and MuLV p15 were again distinguishable in this test. It is interesting to note that an antiserum prepared against a 15,000-molecular-weight protein fraction from F-MuLV (22), obtained from D. Bolognesi, reacted with p15(E) and p15 of both viruses (Fig. 5C and F) and also precipitated viral glycoproteins. These results in Fig. 5 imply an association of both viral glycoproteins with p15(E), but not p15, in the NP-40 lysate of the virions; an association of either viral glycoproteins or p15(E) with p15 is ruled out by the results with anti-R-MuLV p15 serum (Fig. 5B and E). The component designated "x" accompanied precipitates formed by all the MuLV protein antisera used (including others not shown, e.g., anti-p30 and anti-p12), demonstrating a strong adsorptive property but no serological specificity for this protein in these systems, consistent with its being host-cell derived.

DISCUSSION

Our results indicate that MuLV virions contain an additional small structural protein, bringing the total number of structural proteins in these viruses to seven for both mammalian and avian isolates. We have chosen to designate this protein as MuLV p15(E), in distinction to the previously described MuLV p15 (1, 11, 18), since under some circumstances both of these polypeptides appear to have similar molecular weights by gel filtration in the presence of 6 M GuHCl. The latter type of interpretation would account for the results reported by Schäfer et al. (22); whereas under our conditions p15(E) appeared in the void volume fraction, their sample preparation conditions for gel filtration apparently resulted in the recovery of immunogenic amounts of both p15 and p15(E) in a fraction of the elution profile corresponding to a molecular weight of 15,000. Consistent with this is the fact that the antiserum which they prepared against this fraction reacts with both p15 and p15(E) of M- and F-MuLV in the experiment shown in Fig. 5 and predominantly with p15(E) of G-MuLV in an experiment like that in Fig. 4 (E. Tress and E. Fleissner, unpublished data). It is important to note that the p15(E) component precipitated by their serum cannot be due to association with p15 in the NP-40 viral lysate, since p15 is recovered without p15(E) by the use of our anti-R-MuLV p15 serum (Fig. 5). Rather than make a distinction in nomenclature on the basis of a potential small difference in molecular weights of p15 and p15(E), it seems simpler to adopt an additional convention which has been recently proposed (1). This relies on the observation that the *group-specific* p15(E) appears to be situated on the viral *envelope* (16), whereas the *type-specific* p15 is a virion *core* protein (3, 4, 24). (In tests similar to those in Fig. 5 we have shown that the type-specific protein of similar molecular weight recently isolated by Strand and August [23] corresponds to our p15.) Thus the location of p15(E) is denoted by the capital letter suffix, in the manner already used for the MuLV core protein p30(C), and the major polypeptide of the viral nucleoprotein, p10(N) (8).

As demonstrated by Ihle et al. (16), p15(E) is identical to an MuLV protein reactive with normal mouse sera, which was originally reported to have a molecular weight of 17,000 in an SDS-PAGE system. The co-migration of this protein from AKR virus with a radioiodinated F-MuLV protein, which these authors report, is explicable on the basis that the major protein in their preparation is actually F-MuLV p15(E), with very minor amounts of p15 (D. Bolognesi, personal communication).

Figure 5 indicates an association of viral glycoproteins with p15(E) in NP-40-lysed virus. In terms of existing data it is not possible to state whether our anti-void volume serum is reacting predominantly with the gp45 protein component, or with p15(E), or with both components of such a complex. However this interpretation does not affect the two principal conclusions that in our assays p15(E) is quantitatively precipitated in the presence of specific antisera and that it is a virus-specific protein. The latter conclusion is supported by our finding that the antiserum to F-MuLV p15(E) precipitates p15(E) from all MuLVs tested.

This serum, prepared against a low-molecular-weight protein fraction free of glycoprotein (22), produces bright fluorescence in tests with virus-infected mouse embryo cells, but not with uninfected cells (unpublished data).

A protein species with a slightly lower mobility than p15(E), originally identified as "p19" by Ihle et al. (15), is seen in some PAGE patterns (Fig. 4; reference 26). This component is labeled in an identical manner to p15(E) itself by pairs of differentially labeled amino acids (e.g., a met/lys ratio of 0.17; cf. Table 2). From this result we infer a primary structural relationship to p15(E), perhaps reflecting two conformational states of the same molecule, even in SDS buffers. (The fact that this protein aggregates so strongly in 6 M GuHCl suggests some novel structural features.)

It was originally reported that, as in the case of avian leukemia virus, the isolated p15 and p10 proteins of mammalian oncornaviruses co-migrated in SDS-PAGE (18). This erroneous conclusion was arrived at for three reasons: (i) a tendency of p10 to aggregate in PAGE after isolation by the GuHCl-gel filtration procedure and dialysis, particularly in the absence of carrier protein (9); (ii) the as yet unexplained variability in appearance of a p10 peak in certain PAGE profiles of total MuLV proteins (cf. Fig. 3); and (iii) use of PAGE gels of insufficient resolving power. These problems have been more effectively resolved in the present study, which revealed that for a number of endogenous MuLV isolates p15 and p12 virtually co-migrated under our conditions. For several such viruses inversion in the position of these two species relative to their positions in the gel filtration was noted (e.g., Fig. 1 and 2). These results are comparable to those previously reported for another endogenous MuLV isolate (21). As in our original study (7), the advantages of using both gel filtration and PAGE for analysis of viral proteins are evident in the work reported here.

The demonstration of seven structural proteins in MuLV virions, like the reported identification of a small amount of a seventh structural protein in feline leukemia virus with PAGE migration properties similar to MuLV p15(E) (10), removes one of the prima facie differences between the protein compositions of avian and mammalian oncornaviruses: the apparent absence of a mammalian viral protein homologous to avian leukemia virus p19 (18, 19). Whether p15(E) itself is this homologue is unclear; relative protein mobilities in PAGE are not a reliable criterion for analogous functions, but the most economical hypothesis at this time is that the four smallest proteins in both types of viruses in some fashion play similar roles in virion architecture.

ACKNOWLEDGMENT

We are indebted to D. Bolognesi and to M. Strand and J. T. August for providing us with samples of antiserum.

This work was supported in part by Public Health Service grants CA-08748 and CA-16559 from the National Cancer Institute. H. Ikeda is a Special Fellow and W. Hardy is a Scholar of the Leukemia Society of America.

LITERATURE CITED

1. August, J. T., D. P. Bolognesi, E. Fleissner, R. V. Gilden, and R. C. Nowinski. 1974. A proposed nomenclature for the virion proteins of oncogenic RNA viruses. Virology **60**:595–600.
2. Bolognesi, D. P. 1974. Structural components of RNA tumor viruses. *In* M. A. Lauffer, F. B. Bang, K. Maramorosch, and K. M. Smith (ed.), Advances in virus research, vol. 19. Academic Press Inc., New York.
3. Bolognesi, D. P., R. Luftig, and J. H. Shaper. 1973. Localization of RNA tumor virus polypeptides. I. Isolation of further virus structures. Virology **56**:549–564.
4. Buchhagen, D. L., O. Stutman, and E. Fleissner. 1975. Chromatographic separation and antigenic analysis of proteins of the oncornaviruses. IV. Biochemical typing of murine viral proteins. J. Virol. **15**:1148–1157.
5. Duesberg, P. H., K. Beemon, M. Lai, and P. K. Vogt. 1974. *In* W. Robinson and C. F. Fox (ed.), Mechanisms of virus disease, ICN-UCLA symposia in molecular and cellular biology. W. A. Benjamin, Inc., Menlo Park, Calif.
6. Eagle, H. 1959. Amino acid metabolism in mammalian cell culture. Science **160**:432–437.
7. Fleissner, E. 1971. Chromatographic separation and antigenic analysis of proteins of the oncornaviruses. I. Avian leukemia-sarcoma viruses. J. Virol. **8**:778–785.
8. Fleissner, E., and E. Tress. 1973. Isolation of a ribonucleoprotein structure from oncornaviruses. J. Virol. **12**:1612–1615.
9. Fleissner, E., H. Ikeda, J.-S. Tung, E. Vitetta, E. Tress, W. Hardy, Jr., E. Stockert, E. A. Boyse, T. Pincus, and P. O'Donnell. 1975. Characterization of murine leukemia virus-specific proteins. Cold Spring Harbor Symp. Quant. Biol. **39**:1057–1066.
10. Graves, D. C., and L. F. Velicer. 1974. Properties of feline leukemia virus. I. Chromatographic separation and analysis of the polypeptides. J. Virol. **14**:349–365.
11. Green, R. W., D. P. Bolognesi, W. Schäfer, L. Pister, G. Hunsmann, and F. De Noronha. 1973. Polypeptides of mammalian oncornaviruses. I. Isolation and serological analysis of polypeptides from murine and feline C-type viruses. Virology **56**:565–579.
12. Hardy, W. D., Jr. 1971. Immunodiffusion studies of feline leukemia and sarcoma. J. Am. Vet. Med. Assoc. **158**:1060–1069.
13. Hartley, J. W., and W. P. Rowe. 1975. Clonal cell lines from a teral mouse embryo which lack host-range restrictions tor murine leukemia viruses. Virology. In press.
14. Hartley, J. W., W. P. Rowe, and R. J. Huebner. 1970. Host-range restrictions of murine leukemia viruses in mouse embryo cell cultures. J. Virol. **5**:221–225.
15. Ihle, J. N., M. G. Hanna, Jr., L. E. Roberson, and F. T. Kenney. 1974. Autogenous immunity to endogenous RNA tumor virus. Identification of antibody reactivity to select viral antigens. J. Exp. Med. **139**:1568–1581.

16. Ihle, J. N., M. G. Hanna, Jr., W. Schäfer, G. Hunsmann, D. P. Bolognesi, and G. Huper. 1975. Polypeptides of mammalian oncornaviruses. III. Localization of p15 and reactivity with natural antibody. Virology **63**:60–67.
17. Ikeda, H., T. Pincus, T. Yoshiki, M. Strand, J. T. August, E. A. Boyse, and R. C. Mellors. 1974. Biological expression of antigenic determinants of murine leukemia virus proteins gp69/71 and p30. J. Virol. **14**:1274–1280.
18. Nowinski, R. C., E. Fleissner, N. H. Sarkar, and T. Aoki. 1972. Chromatographic separation and antigenic analysis of proteins of the oncornaviruses. II. Mammalian leukemia-sarcoma viruses. J. Virol. **9**:359–366.
19. Nowinski, R. C., N. H. Sarkar, and E. Fleissner. 1973. Isolation of subviral constituents and antigens from the oncornaviruses, p. 237–285. *In* H. Busch (ed.), Methods in cancer research, vol. VII. Academic Press Inc., New York.
20. Old, L. J., E. A. Boyse, and E. Stockert. 1964. Typing of mouse leukemias by serological methods. Nature (London) **201**:777–779.
21. Pal, B. K., M. Wright, J. E. Officer, M. B. Gardner, and P. Roy-Burman. 1973. Subviral components of a wild mouse embryo-derived type C oncornavirus. Virology **56**:189–197.
22. Schäfer, W., G. Hunsmann, V. Moennig, F. de Noronha, D. P. Bolognesi, R. W. Green, and G. Huper. 1975. Polypeptides of mammalian oncornaviruses. II. Characterization of a murine leukemia virus polypeptide (p15) bearing interspecies reactivity. Virology **63**:48–59.
23. Strand, M., and T. August. 1973. Structural proteins of oncogenic ribonucleic acid viruses. Interspec, II, a new interspecies antigen. J. Biol. Chem. **248**:5627–5633.
24. Strand, M., R. Wilsnack, and J. T. August. 1974. Structural proteins of mammalian oncogenic RNA viruses: immunological characterization of the p15 polypeptide of Rauscher murine leukemia virus. J. Virol. **14**:1575–1583.
25. Weissmann, C., J. T. Parsons, J. W. Coffin, L. Rymo, M. A. Billeter, and H. Hofstetter. 1975. Studies on the structure and synthesis of Rous sarcoma virus RNA. Cold Spring Harbor Symp. Quant. Biol. **39**:1043–1056.
26. Witte, O. N., and I. L. Weissmann. 1974. Polypeptides of Moloney sarcoma-leukemia virions: their resolution and incorporation with extracellular virions. Virology **61**:575–587.