Properties of Feline Leukemia Virus

I. Chromatographic Separation and Analysis of the Polypeptides¹

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Rickard's strain of feline leukemia virus (FeLV) contains two large glycoproteins and five smaller polypeptides of molecular weights 100,000 (gp $\geq$ 100), 70,000 (gp70), 30,000 (p30), 21,000 (p21), 15,000 (p15), 11,200 (p11), and 10,000 (p10) when chromatographed on 6% agarose in the presence of 6 M guanidine hydrochloride (GuHCl). P21 is a minor component which was not previously described for mammalian leukemia-sarcoma viruses and may be analogous to the seventh protein found in avian viruses. Analysis on 4% agarose and by sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed that gp $\geq$ 100 is actually $\geq$ 200,000 daltons and dissociates to polypeptides of approximately 100,000 to 115,000 daltons, whereas gp70 can be resolved into six stained bands ranging from 40,000 to 80,000 daltons despite being rechromatographed as a single symmetrical peak on 6% agarose. Rechromatography on 8% agarose was found to be more effective than on 6% agarose or sodium dodecyl sulfate polyacrylamide gel electrophoresis for obtaining the five small polypeptides, especially p11 and p10, in a highly purified form suitable for further analysis and for obtaining more precise estimates of their molecular weights, especially when done by co-chromatography with iodinated standard proteins markers. Rechromatographed p30, p21, p15, p11, and p10 had molecular weights of 27,000, 18,000, 15,000, 12,000, and 12,000 respectively, by co-electrophoresis with the marker proteins on sodium dodecyl sulfate polyacrylamide gel electrophoresis, clearly establishing that the latter two FeLV polypeptides comigrate to form one less band when compared to elution from agarose. The isoelectric points of p30 and p15 were 5.5 and 8.9, respectively, after renaturation from GuHCl and 5.6 and 8.7, respectively, when isolated from Tween-ether treated virus. Rechromatographed p30, p15, and p11, renatured by removing GuHCl with dialysis, reacted only with their homologous antisera in immunodiffusion analysis, indicating that they are immunologically unrelated. Also the interspecies gs-3 determinant associated with p30 could be regained by removal of GuHCl.

Early separation of avian (1, 3, 16) and mammalian (8, 22, 29, 38) oncornavirus structural polypeptides, including those of feline leukemia virus (FeLV), depended largely on analysis by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS). With SDS-PAGE it was possible

to resolve six and five proteins from the avian and mammalian viruses respectively, but there were frequent problems with poor recovery of antigenic activity. By using various extraction and isolation techniques, the major protein components from C-type viruses have been studied (1, 3, 13, 16, 37, 38, 40) with the major protein (about 30,000 molecular weight) of murine leukemia virus (MuLV), and to some extent FeLV, being analyzed most extensively (2, 11, 12, 24, 28, 29, 30, 31, 37, 38). This emphasis was based on the relative abundance of the proteins in the virions and the fact that they are the major group-specific (gs) antigens which carry the interspecies antigen (9, 10, 11), also known as the gs-3 (9) or gs-interspec (39, 39, 40)

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antigen. The characteristics reported for the FeLV gs antigen were variable (2, 30, 38, 41), probably due to differences in homogeneity resulting from variations in the techniques used to isolate and assay this protein (2).

Recently, gel filtration on 6% agarose in the presence of 6 M guanidine hydrochloride (GuHCl) and a reducing agent (6) was used to resolve seven and six proteins from avian (7) and mammalian (23) viruses, respectively. In each case the higher resolution in the low molecular weight range allowed the identification of a small protein not previously described on SDS-PAGE. Furthermore, dialysis of separated avian and murine proteins to remove GuHCl permitted efficient renaturation and recovery of antigenic activity (7, 23). Gel filtration on agarose in GuHCl (GuHCl-agarose) was also used to separate six FeLV polypeptides but further purification and analysis of these components was not done (23). In light of the important role of feline oncornaviruses in comparative studies (25-27) and as a model system for understanding viral oncogenesis (14) it seemed worthwhile to analyze the FeLV polypeptides as thoroughly as the other oncornaviruses. In addition to the gs antigen, which was already partially characterized, the other five FeLV polypeptides may be essential virion structural components and they also deserved thorough analysis. In this study several small FeLV polypeptides were purified, characterized, and shown to be very similar to the proteins of other mammalian viruses (23). The purpose of this communication is to report new information about these small polypeptides and FeLV glycoproteins which either increases or clarifies our knowledge of mammalian oncornavirus polypeptides in general and extends the usefulness of the GuHCl-agarose method.

MATERIALS AND METHODS

Source of virus. The chronically infected feline thymus tumor cell suspension, F-422, which was derived from a neoplastic thymus of leukemia kitten, was used as the source of virus (34). This strain of FeLV, known as the Rickard's strain, was obtained from C. G. Rickard (Cornell University). For optimal growth, cells were propagated in suspension cultures by using a complex growth medium consisting of 40% McCoy's 5A and 60% Leibovitz L-15 plus 15% heat-inactivated fetal calf serum (GIBCO). These cells were passed twice each week at 3- and 4-day intervals by dilution to 2×10^5 or 10^5 cells per ml, respectively. Under these conditions the cells doubled every 24 h to cell densities of 2×10^6 cells per ml or more with greater than 90% viability. Approximately 3 to 5 mg of viral protein can be obtained from each liter of culture medium produced in this manner.

Radioactive labeling of virus. Virus was labeled

by including radioactive precursors (obtained from New England Nuclear Corp., Boston, Mass.) into the culture medium of the F-422 cells. Virus labeled with either [^3H]uridine (26 Ci/mmol) or [^3H]glucosamine (D-glucosamine, 36 Ci/mmol) was prepared by addition of 0.5 to 2 $\mu\text{Ci/ml}$ or 5 $\mu\text{Ci/ml}$, respectively, to growing cells (approximately 2×10^6 cells per ml) that had doubled in number during the previous 24 h of incubation in fresh growth medium. Virus labeled with ^{14}C amino acids was prepared by the addition of 0.5 μCi of ^{14}C amino acid mixture per ml to cells grown as described above and also brought to a concentration of 2×10^6 cells per ml. However, in this case the cells were pelleted from normal growth medium and resuspended in a modified medium containing 10% of the normal level of amino acids and 10% heat-inactivated fetal calf serum to increase the level of radioactive amino acid incorporation. With all three isotopes the cultures were incubated for 24 to 48 h at 37 C, after which they were harvested and the medium containing radioactive virus was used for virus purification.

Purification of virus. Unlabeled virus was purified from the culture medium by a modification of the original method of Robinson et al. (35). The main modification involved the use of 5% (wt/vol) polyethylene glycol (PEG, Carbowax 6000, Union Carbide) instead of ammonium sulfate to precipitate and concentrate the virus (21, 32). PEG was made up as a 50% (wt/vol) solution in 0.14 M NaCl and one part of 50% PEG was added to nine parts of culture medium. Virus precipitation was accomplished by stirring at 4 C for as short as 90 min to as long as 18 h. The precipitate was collected by centrifugation in a Sorvall GSA rotor at 9,000 rpm for 30 min. The precipitated and pelleted virus was then carefully drained and resuspended in TNE buffer containing 5% sucrose to achieve a final concentration of 1% of the original volume. The use of PEG permitted a 100-fold concentration of virus rather than the 10-fold concentration obtained with ammonium sulfate, and it became feasible to purify virus from 12 liters of culture medium in one purification procedure by using an SW 27 rotor (Beckman Instruments). Further modifications include the use of three sucrose gradients (one discontinuous and two linear) of 20 to 50% sucrose (wt/vol) in TNE buffer (0.01 M Tris, pH 7.4, 0.1 M NaCl, 0.001 M EDTA). The discontinuous gradient consisted of 10 ml of 50% sucrose in TNE, 8 ml of 20% sucrose in TNE, and 20 ml of resuspended virus. The gradients were centrifuged for 4 h at 25,000 rpm in the SW 27 rotor and the visible virus band was collected from each gradient by puncturing the bottom of the tube. The virus obtained from the last linear gradient was dialyzed against TNE buffer to remove the sucrose, assayed for protein content, and then stored at -70 C until used.

In several early experiments, radioactively labeled virus was purified by a small-scale PEG precipitation procedure. More recently, ^{14}C -amino-acid-labeled virus was purified in a somewhat different manner because of the smaller volumes of culture medium to be processed. Instead of precipitating the virus from the culture medium with PEG, it was pelleted in an SW 27 rotor at 25,000 rpm for 75 min at 4 C. The viral

pellets were then resuspended in a small amount of TNE buffer and loaded onto a 5-ml linear gradient of 20 to 50% (wt/vol) sucrose in TNE buffer and centrifuged for 2.5 h at 50,000 rpm in the SW 50.1 rotor (Beckman Instruments, Inc.). The visible virus band was collected, diluted with TNE buffer, and resedimented once on another linear sucrose gradient. The final visible virus band was then collected, dialyzed in TNE buffer, and stored at -70°C until used.

Determination of protein content. Protein concentrations were determined according to the procedure of Lowry et al. (18) by using crystalline bovine serum albumin as the standard.

Iodination. The technique of Helmkamp et al. (15) using iodine monochloride was employed for iodination of a minor protein component (p21) to facilitate analysis. After iodination, the protein was dialyzed extensively with several changes of TNE buffer to remove free iodine. The same method (15) was used to iodinate the marker proteins (transferrin, ovalbumin, chymotrypsinogen, hemoglobin, and cytochrome *c*) which were then used for molecular weight determinations. After iodination, the marker proteins were chromatographed on G-200 Sephadex to remove free iodine and to separate single protein molecules from aggregates.

Gel filtration analysis of viral proteins in GuHCl. For separation of large amounts of viral protein a mixture of unlabeled virus (10 to 20 mg of protein) and sufficient ^{14}C -amino-acid-labeled marker virus (0.15×10^5 to 1.5×10^5 counts/min, usually 10^5 counts/min) were pelleted by centrifugation for 1 h at 25,000 rpm. In several experiments [^3H]glucosamine-labeled virus was included with the ^{14}C -labeled and unlabeled virus. The virus pellet was solubilized in 0.5 ml of 8 M guanidine-hydrochloride (GuHCl; Heico, Inc., Delaware Water Gap, Pa.) containing 2% mercaptoethanol or 0.05 M dithiothreitol (DTT), 0.01 M EDTA, and 0.05 M Tris (pH 8.5) at 56°C for 45 min as described by Fleissner (7). After the incubation, 0.1 ml of eluant buffer (6 M GuHCl, 0.01 M DTT, and 0.02 M sodium phosphate, pH 6.5) containing 0.6% blue dextran and 50% sucrose was added to the viral protein solution and the protein solution was loaded onto a 6% agarose (Bio-Gel A-5M, 200–400 mesh, Bio-Rad, Richmond, Calif.) column (90 by 1.2 cm) and chromatographed in a manner similar to the method of Fish et al. (6) and Fleissner (7). Columns were run at room temperature at a flow rate of approximately 1 ml per h with 1-ml fractions being collected. Portions of each fraction were assayed for radioactivity as described below.

After the initial chromatography in 6% agarose, the effluent fractions from individual radioactive peaks were pooled and concentrated to a volume of 0.5 to 1.0 ml by negative pressure dialysis against eluant buffer with 8 M rather than 6 M GuHCl. Powdered sucrose was then added to a concentration of 10% (wt/vol) and the material was rechromatographed on either 6 or 8% (Biogel A 1.5 M, Bio-Rad, 200 to 400 mesh) agarose columns as described above. After rechromatography of proteins twice, peak fractions from individual proteins were pooled, fresh DTT was added (0.01 M), and the protein was dialyzed extensively in the cold against a low ionic strength buffer (2 mM Tris, pH

7.4) to remove GuHCl. The protein samples were then stored in 2-ml samples at -70°C until used.

Initially, work with 4% agarose (Biogel A 15 M, Bio-Rad, 200 to 400 mesh) was with large columns run exactly as described above except that the flow rate was much slower (0.5 ml per h or less). The one experiment with a 4% column presented in this paper was done on a small column developed to facilitate more economical analytical gel filtration when preparative size columns are not needed. The small column with a 5.75-fold lower volume can be used with 6 and 8% agarose as well and is operated as described above with the following modifications. An agarose column (90 by 0.5 cm) was poured in a 100-cm length of heavy walled glass tube (8 mm outside diameter, 5 mm inside diameter) of the type used for polyacrylamide gel electrophoresis. The tube was sealed at the bottom end with a rubber tube cap for gel electrophoresis (Buchler Instruments, Fort Lee, N. J.), lined with glass wool and penetrated by a cut off 20-gauge hypodermic needle to permit elution. The sample was prepared as above except that the final volume was kept to 0.15 ml or less and was loaded with a microliter syringe. The fraction size was reduced proportionately to approximately 0.18 ml, fractions were collected every hour, and the collection tubes were stoppered every 12 h to minimize evaporation. Then 0.15 ml or nearly all of each fraction was assayed for radioactivity as described below. When the flow rate was kept low so the small fractions were collected every hour or less the degree of separation of protein peak was equivalent to that obtained on the larger columns. Although this small scale column could not be run faster than a large one, analytical runs could be done much more economically and entire fractions could be processed for assay of radioactivity with much less difficulty.

PAGE. Two different procedures were used for PAGE in the presence of 0.1% SDS. Initially the neutral-SDS gel electrophoresis procedure using phosphate buffer and no spacer gel was carried out as described by Maizel (19) by using a 7.5% gel. One hundred microliter samples of rechromatographed gp ≥ 100 and gp70 or purified FeLV (25 to 100 μg of protein) were prepared as described (19), and loaded onto 10-cm gels that had been pre-run for 1 h. Electrophoresis was at 2.0 mA/gel for 30 min and then at 3 mA/gel until the dye reached end of the gel (15 h). The gels were fixed overnight in 20% sulfosalicylic acid, stained with Coomassie brilliant blue R250 for 3 h followed by repeated washes with 7% acetic acid. When thoroughly destained, the gels were scanned in a Gilford spectrophotometer with linear transport at 570 nm.

More recently, the procedure of PAGE on 10-cm 7.5 and 10% polyacrylamide gels with a 1-cm spacer gel of 3% polyacrylamide was used as described by Maizel (19). Twenty-five to 100- μliter samples of either purified FeLV or rechromatographed low molecular weight polypeptides (25 to 100 μg of protein for staining, 2×10^3 to 10×10^3 counts/min ^{14}C -amino-acid-labeled polypeptides or 2×10^3 to 4×10^3 counts/min of iodinated p21 for slicing and counting) were loaded on top of 10-cm gels. Electrophoresis was carried out at a constant voltage of 100 V at room

temperature for 4 to 6 h. When gels prepared by this procedure were to be stained, they were removed from the gel tubes and stained with Coomassie blue (0.25%) in 50% methanol and 7% acetic acid for 1 to 2 h. The gels were then electrophoretically destained in 7% acetic acid for 15 to 30 min. They were then scanned with a Gilford spectrophotometer at 570 nm.

Polyacrylamide gels to be assayed for radioactivity were fractionated on a Gilson gel fractionator (Gilson Medical Electronics, Inc.) into 1-mm fractions which were minced and flushed directly into scintillation vials for counting, as described below.

Radioactive assay. Counting of all ^3H - and ^{14}C -labeled samples was carried out in a Packard Tricarb liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.). Samples (0.01 to 0.2 ml) of fractions obtained from either agarose columns, preparative sucrose gradients, or isoelectric focusing columns were spotted on 2.3-cm diameter Whatman 3MM filter disks and dried thoroughly at 60 C and prepared for counting as previously described (47, 48) except that the hot trichloroacetic acid step was eliminated. Minced fractions from polyacrylamide gels were solubilized by shaking with 5 ml of Aquasol (New England Nuclear Corp., Boston, Mass.) for 10 min and then counted. Counting of ^{125}I -labeled samples was carried out in a Packard autogamma spectrometer (Packard Instrument Co.). The processing of samples and correction for ^{125}I cross-over in the ^{14}C channel during double label experiments were carried out as previously described (48).

Tween-ether disruption of FeLV. A suspension of FeLV (0.5 to 1.2 mg of protein per ml) was treated with 0.2% Tween 80 (Nutritional Biochemicals, Cleveland, Ohio) and 4 to 5 volumes of anaesthetic grade ether and processed as in procedure A of Oroszlan et al. (28). The material was stored at -20 C until used.

Isoelectric focusing. Purified ^{14}C -amino-acid-labeled and unlabeled FeLV preparations were mixed (total counts approximately 10^5 counts/min, total protein approximately 2 mg), treated with Tween 80-ether (as described above) and were digested with solid RNase at 0.2 mg (Enzite-RNase, insolubilized enzyme, Miles Laboratory, Inc., Elkhart, Ind.) and 20 μg of bovine pancreatic DNase per ml (Worthington, Freehold, N. J.) at 37 C for 20 min to eliminate nucleic acids which might bind to proteins and alter their isoelectric point (30). Aggregates of subviral components, undisrupted virus (if any), and RNase were removed by centrifugation at 50,000 rpm at 4 C in the SW 50.1 rotor for 1 h before isoelectric focusing. Proteins obtained in this manner or from agarose column were dialyzed and isoelectric focused in a 110-ml capacity glass column (model 1801, LKB Instruments, Inc., Rockville, Md.) as described in the manufacturer's instructions with the cathode on the bottom except that 5% sucrose was used in the light solution. Other experimental details were as described by Oroszlan et al. (28, 29). The voltage was increased to a maximum of 700 V and held until the amperage remained constant for at least 12 h. Care was taken not to exceed 1.5 W at any time. At the end of the focusing period (usually 48 to 60 h), 1.5- to 2.0-ml fractions (55 to 80) were collected from the

bottom of the column and assayed for pH (Radiometer, London Co., Cleveland, Ohio) and for radioactivity as described in a previous section. Fractions making up radioactive peaks were stored at -20 C and analyzed in other experiments.

Estimation of molecular weights of viral polypeptides. Estimation of molecular weights of the viral polypeptides was carried out by electrophoresis in 7.5 and 10% SDS-polyacrylamide gels (19) according to the method of Shapiro et al. (42), and on agarose columns in GuHCl by the method of Fish et al. (6) using the following iodinated protein standards: cytochrome *c* (molecular weight 12,400), hemoglobin (molecular weight 16,000), chymotrypsinogen (molecular weight 25,700), ovalbumin (molecular weight 43,000), and human transferrin (molecular weight 76,600). These markers were obtained from Sigma Chemical Co., St. Louis, Mo.) and were iodinated as described previously. Iodinated marker proteins were mixed with the rechromatographed ^{14}C -amino-acid-labeled viral polypeptides and coelectrophoresed or cochromatographed on SDS-PAGE or agarose columns, respectively. Standard curves were constructed by plotting the distance each marker protein migrated in SDS gels or the elution position from GuHCl columns versus the logarithm of the respective molecular weights. The molecular weights of the viral proteins were determined by extrapolating from the standard curve.

Sucrose gradient analysis of isolated polypeptides. Isolated viral polypeptides were treated with 1 M GuHCl to minimize aggregation and were analyzed on 4.8-ml linear gradients of 5 to 20% (wt/vol) sucrose in TNE buffer at pH 7.4 and containing 1 M GuHCl. Isolated polypeptides (3,000 to 9,000 counts/min of ^{14}C) to be used for analysis were prepared in final volumes of 0.5 ml containing iodinated bovine serum albumin (BSA) or iodinated chymotrypsinogen as markers (3,000 to 10,000 counts per min per gradient). The gradients were centrifuged for 24 to 40 h at 50,000 rpm in the SW 50.1 rotor at 4 C. Approximately 35 fractions (0.14 ml) were collected through the bottom of the tube onto filter disks and the disks were processed and counted for ^{125}I and ^{14}C radioactivity as described. Approximate sedimentation coefficients were calculated by the method of Martin and Ames (20), assuming a value of 4.4S for BSA (17), and of 2.54S for chymotrypsinogen (36, 43).

Preparation of antisera. New Zealand White or Dutch belted rabbits were inoculated in each of their four foot pads with Tween 80-ether-treated FeLV preparation (2 ml total emulsion containing 1 mg of protein) or purified proteins (2 ml total emulsion containing approximately 5 μg of protein p11, p10, p21, or 200 μg of protein p30 or p15) in complete Freund adjuvant (equal volume adjuvant and protein solution). Subsequent injections of equal amounts of protein solution emulsified with incomplete Freund adjuvant were given subcutaneously at four sites 20 days after the initial immunization. The rabbits were ear bled 10 days after the second injection, and the sera were checked for precipitating antibody by immunodiffusion analysis. Rabbits exhibiting precipitating antibody were bled by cardiac puncture at 48-h intervals. Rabbits that did not exhibit precipitating

antibody after the second injection were given subsequent injections every 10 days for three to four times with the respective protein (except for the limited p21) emulsified with incomplete Freund adjuvant as described. Rabbit antiserum prepared against rechromatographed protein p30 from Rauscher murine leukemia virus (MuLV p30) was obtained from Erwin Fleissner and William Hardy (Sloan Kettering Institute, N. Y.).

Immunodiffusion analysis. Double diffusion (Ouchterlony) analyses were performed on slides of 2% Noble agar (Difco, Detroit, Mich.) in phosphate-buffered saline (PBS, 0.01 M phosphate, 0.85% NaCl, pH 7.2) with merthiolate (1 part in 10,000) as a preservative. The slides were stored at room temperature in a moist chamber and were evaluated and photographed after optimal precipitation had occurred (usually by 24 h).

RESULTS

Separation of viral polypeptides by gel filtration on guanidine hydrochloride. A mixture of purified unlabeled FeLV containing 10 mg of protein and ^{14}C amino-acid- and ^3H glucosamine-labeled virus was analyzed on 6% agarose gel in the presence of 6 M GuHCl. The elution profile of the ^{14}C -amino-acid-labeled proteins (Fig. 1A) indicates that the expected six major proteins and one previously unreported minor protein were resolved from FeLV. Initially, only six major proteins were detected, but more recently the minor component was consistently observed when good separation of peaks was achieved on 6% agarose under optimal conditions (Fig. 1A). In fact, the minor peak was usually more apparent than shown but the profile in Fig. 1A was included for the glucosamine data. Analysis of [^3H]glucosamine revealed that the first two proteins eluting from the column contain carbohydrate and were considered glycoproteins, whereas the remaining smaller proteins appeared to be free of significant carbohydrate as determined under the conditions of the experiment and were considered as nonglycoproteins (Fig. 1A).

The polypeptides are identified according to a new nomenclature^a that is based on their species origin (Fe for feline), type of virus (LV for leukemia virus), whether the protein is a glycoprotein (gp) or not (p), and their molecular weight by the method of choice (gel filtration on agarose in GuHCl in this case). The FeLV polypeptides are designated as: FeLV gp ≥ 100 (eluted with the void volume on 6% agarose), FeLV gp70, FeLV p30, FeLV p 21, FeLV p15,

FeLV p11, and FeLV p10 (Table 1). Since FeLV was the only virus used in this study the species and virus designation will be dropped for the remainder of this paper. Initially approximate molecular weight data was obtained by calibrating the preparative 6% agarose column with marker proteins eluted under similar conditions. The nomenclature for gp ≥ 100 and gp70 is based on this preliminary data and is included in Table 1 for completeness. The more accurate molecular weight data for the five low molecular weight polypeptides is based on more rigorous cochromatographic analysis of marker proteins and individual rechromatographic polypeptides as described later. Comparison of the gel filtration data in Fig. 1A with the electropherogram pattern (Fig. 2D) characteristic of C-type viruses (8, 22) shows that better separation of lower molecular weight proteins was obtained with GuHCl-agarose than with SDS-PAGE, and this was especially important in the case of p11 and p10 and also p21. Because of this better resolution, high recovery and the ease of renaturing the small proteins (except for p15) it was decided to use agarose chromatography as a preparative technique for obtaining separated FeLV proteins.

Rechromatography and SDS-PAGE analysis of the two glycoproteins. Further separation of gp ≥ 100 and gp70 was attempted by rechromatography of 6% agarose (Fig. 2A and B). Both proteins rechromatographed as single peaks free from each other with gp ≥ 100 still eluting in the void volume (Fig. 2A). Preliminary analysis of these single rechromatographed peaks in 7.5% polyacrylamide gels in the presence of SDS revealed that they were heterogeneous and could be resolved into several polypeptide components. Gp70 gave six visible bands in the molecular weight region of 40,000 to 80,000 (Fig. 2C) and none coincided with the lower molecular weight proteins of the virus (Fig. 2D). Two polypeptides (identified as 2 and 3) coincided exactly with the two major high molecular weight proteins seen in whole virus (Fig. 2D). With gp ≥ 100 , several poorly separated stained polypeptide bands were found in the approximate molecular weight region of 110 to 115,000 at the top of the gel and none in the intermediate and low molecular weight region (Table 1, L. Velicer, unpublished preliminary observations). These preliminary experiments with gp ≥ 100 were done on 7.5% gels with marker proteins no larger than transferrin (76,000 daltons) and accurate molecular weight estimates were not possible near the top of the gel. For reference, these stained bands from gp ≥ 100 were in the upper 1 cm of gel in the same region of several

^a The nomenclature follows the format recommended by the group of workers attending the oncornavirus antigen workshop at the Sloan Kettering Institute for Cancer Research in New York City, June 4 and 5, 1973, in an effort to establish a consistent, flexible, universal means of identification.

FIG. 1. Gel filtration analysis of FeLV polypeptides from a mixture of virus labeled with ^{14}C amino acids and ^3H glucosamine. (A) The two labeled virus preparations (2.5×10^4 counts/min of ^3H glucosamine and 6.5×10^4 counts/min of ^{14}C amino acids; total protein 10 mg) were mixed, dissociated in 8 M GuHCl, loaded onto a 6% agarose column, and eluted with 6 M GuHCl in the presence of 0.01 M DTT at a rate of 1 ml/h. Eluted fractions (1 ml) were assayed for radioactivity, and the peak fractions from each protein component were pooled and concentrated by negative pressure dialysis as described in Materials and Methods. Each protein is identified by its molecular weight as determined by the method of Fish et al. (6). (B) The two labeled virus preparations (1.6×10^5 counts/min of ^{14}C and 2.5×10^5 counts/min of ^3H) were mixed and chromatographed as in A except that the column contained 4% agarose and the small scale modification was used as described in Materials and Methods.

TABLE 1. *Properties of feline leukemia virus proteins*

Protein species	Percent of total viral protein ^a	Mol wt ^b determined by		Isoelectric points after		Sedimentation coefficients
		GuHCl-Agarose	SDS-PAGE	GuHCl-Agarose	Tween 80-ether	
gp $\geq$ 100	18.4 $\pm$ 0.5	$\geq$ 100,000 ^c	110-115,000 ^d	ND	ND	ND
gp 70	12.2 $\pm$ 1.1	70,000 ^c	40-80,000 ^e	ND	ND	ND
p30	33.8 $\pm$ 0.7	30,000	27,000	5.5	5.6	2.5S
p21	0.7 $\pm$ 0.07	21,000	18,000	ND	ND	ND
p15	19.8 $\pm$ 0.8	15,000	15,000	8.9	8.7	1.4S
p11	8.5 $\pm$ 0.5	11,200	12,000	4.2 ^f	ND	1.17S
p10	6.6 $\pm$ 0.3	10,000	12,000	3.8 ^f	ND	1.14S

^a The percent of each protein was determined from typical ¹⁴C amino acid radioactivity profiles on 6% agarose as seen in Fig. 1A. The total radioactivity of each protein species was divided by the total radioactivity recovered from the column. The data are averages of three separate determinations.

^b Average of at least three determinations.

^c Molecular weight estimates for gp $\geq$ 100 and gp 70 are preliminary values obtained with calibrated 6% agarose columns (Fig. 1A), whereas the estimates for the other proteins were obtained by cochromatography of rechromatographed polypeptides with marker proteins.

^d Several visible bands when stained with Coomassie blue (L. Velicer, unpublished preliminary observations).

^e Six visible bands ranging from 40,000 to 80,000 daltons when stained with Coomassie blue (Fig. 2C).

^f Preliminary data which requires verification with larger amounts of material.

small peaks detected in whole virus (Fig. 2D).

A 4% agarose column, with an exclusion limit of approximately 220,000 daltons in the presence of GuHCl (45) was used to determine if better separation of the large glycoproteins into their polypeptide components could be obtained (Fig. 1B). The two glycoproteins identified by [³H]glucosamine label appear further separated from each other, but they are still single peaks and are not resolved into their components. There seems to be some separation of ¹⁴C-amino-acid-labeled polypeptides in the region of the second glucosamine peak, suggesting that not all the material found in gp70 on 6% columns (Fig. 1A) is glycoprotein. However, the amount of radioactivity assayed was too low to permit a firm conclusion. The higher molecular weight glycoprotein still eluted with the void volume and has a molecular weight of $\geq$ 220,000 instead of $\geq$ 100,000 as determined on 6% agarose. Resolution of the lower molecular weight proteins was very poor on 4% agarose and p11 and p10 were not separated at all.

Rechromatography and SDS-PAGE analysis of the five small polypeptides. An 8% agarose column, with an exclusion limit of approximately 40,000 daltons in the presence of 6 M GuHCl (D. Graves, unpublished data) was used to further separate proteins of low molecular weight. Proteins p11 and p10 which were poorly separated on 6% agarose (Fig. 1A) were better separated on 8% agarose (Fig. 3A). In this particular experiment, peak fractions of p11

and p10 were pooled together after chromatography on 6% agarose (Fig. 1A) and then rechromatographed on an 8% agarose column (Fig. 3A). Further purification of the two proteins on 8% agarose resulted in each protein forming symmetrical peaks well separated from one another (Fig. 3B and 3C).

In preliminary experiments, p30 and p15 obtained from the first 6% agarose column were analyzed by SDS-PAGE and immunodiffusion and found to be contaminated with adjacent proteins despite their better initial separation (Fig. 1A). It was therefore necessary to rechromatograph them also for further separation. P30 was first rechromatographed on 6% agarose (Fig. 4A) and then on 8% agarose (Fig. 4B), whereas p15 was rechromatographed twice on 8% agarose (Fig. 4C and 4D). After being rechromatographed twice, they appeared as homogenous peaks (Fig. 4B and 4D) free of their nearest neighbors. Because of the small amount of p21 available (Table 1) peak fractions from several chromatography columns were pooled and iodinated to facilitate analysis. Figures 4E and F contain profiles of iodinated p21 after it was rechromatographed on 6 and 8% agarose, respectively. Further analysis of this protein was limited because of the small amounts available (Table 1).

To more precisely assess their relative rates of migration, the five isolated low molecular weight polypeptides (p30, p15, p11, p10, p21) that had been chromatographed three times on

FIG. 2. Gel filtration and SDS-PAGE analysis of gp ≥ 100 and gp 70. (A and B) Peak fractions of ^{14}C -labeled gp ≥ 100 and gp 70, respectively, obtained from 6% agarose (Fig. 1A) were pooled, concentrated as described in Materials and Methods, and chromatographed on 6% agarose as in Fig. 1A. (C and D) Rechromatographed gp 70 (B above) and unlabeled FeLV were subjected to electrophoresis in 7.5% gels without a spacer gel containing 0.1% SDS for 15 h at 3 mA after being solubilized in 1% SDS and 1% 2 mercaptoethanol. The method of electrophoresis and the staining of protein bands with Coomassie blue are according to the first procedure described in Materials and Methods. Absorbance profiles of the gels were determined at 570 nm.

FIG. 3. Gel filtration analysis of p11 and p10. ^{14}C -labeled p11 and p10 obtained from 6% agarose (Fig. 1A) were concentrated and chromatographed on 8% agarose, and assayed as in Fig. 1A. (A) Elution profile of p11 and p10 rechromatographed on 8% agarose. (B) Elution profile of p11 after being rechromatographed twice on 8% agarose. (C) Elution profile of p10 after being rechromatographed twice on 8% agarose.

FIG. 4. Gel filtration analysis of p30, p15, and p21. ^{14}C -labeled p30 and p15 obtained from 6% agarose

6 and 8% agarose in the presence of GuHCl were dissociated with 1% SDS and electrophoresed on 10% polyacrylamide gels (Fig. 5). These proteins had relative mobilities in PAGE as follows: p30 migrated as the slowest protein as expected, p21 migrated between p30 and p15 in a manner similar to its elution pattern off of agarose columns, and p11 and p10 migrated in an identical position as expected from preliminary work with whole virus (Fig. 2D). Attempts to fully resolve differentially labeled p11 and p10 by SDS-PAGE with 15% polyacrylamide gels were unsuccessful and resulted in a difference of only one fraction between peak fractions (D. Graves, unpublished observations).

Molecular weight estimation of rechromatographed polypeptides. Analysis of the rechromatographed low molecular weight polypeptides by coelectrophoresis in gels containing SDS (19) and by cochromatography on agarose columns in the presence of 6 M GuHCl (6) along with iodinated protein markers (see Materials and Methods) permitted more precise estimations of their individual molecular weights (Table 1). In SDS-PAGE with 10% polyacrylamide gels, p30 had a molecular weight of

(Fig. 1A) and p21 from the same source but iodinated as described in Materials and Methods, were rechromatographed on 6 and 8% agarose and analyzed as in Fig. 1A and 2. (A) Elution profile of p30 rechromatographed on 6% agarose. (B) Elution profile of p30 rechromatographed on 8% agarose after being rechromatographed once on 6% agarose (A). (C) Elution profile of p15 rechromatographed on 8% agarose. (D) Elution profile of p15 rechromatographed twice on 8% agarose. (E) Elution profile of p21 rechromatographed on 6% agarose. (F) Elution profile of p21 rechromatographed on 8% agarose after being rechromatographed once on 6% agarose (E).

FIG. 5. SDS-polyacrylamide gel electropherogram of individual rechromatographed FeLV proteins. ^{14}C -amino-acid-labeled proteins, (A) p30, (B) p15, (C) p11, (D) p10 and ^{125}I -labeled protein, (E) p21 obtained from agarose columns (Fig. 3 and 4), were analyzed in 10% gels with a 3% spacer gel followed by fractionation and analysis as described in Materials and Methods. The proteins were concentrated to a small volume, solubilized in 1% SDS and 0.1% 2-mercaptoethanol, and boiled for 1 min. A 50- μl sample was applied to a gel and electrophoresed at 100 V for 5 h. Migration is from left to right.

27,000 which was similar to that obtained by others (30); p15 and p21 had molecular weights of 15,000 and 18,000, respectively; and p11 and p10 migrated in an identical region of about 12,000 molecular weight as seen in Fig. 6. Measurement of molecular weights by gel filtration was done by cochromatography on 8% agarose columns and resulted in the following: p30 = 30,000, p21 = 21,000, p15 = 15,000, p11 = 11,200, and p10 = 10,000 daltons (Table 1). A comparison of the molecular weights obtained by gel filtration in the presence of GuHCl with that of SDS-PAGE revealed the following (Table 1): p30 and p21 had relatively higher molecular weights in GuHCl than they did in SDS; p15 had identical molecular weights on both systems; and p11 and p10 had slightly greater molecular weights by SDS-PAGE.

Isoelectric focusing of the FeLV proteins and rechromatographed polypeptides. To further analyze the FeLV proteins, ^{14}C -amino-acid-labeled proteins from Tween 80-ether disrupted virus and polypeptides obtained by rechromatography on GuHCl-agarose were analyzed by isoelectric focusing. Two distinct major peaks were repeatedly observed with Tween 80-ether disrupted virus, one at a pH of 8.7 and one at a pH of 5.6 (Fig. 6A). In addition, a broad minor peak of radioactivity was regularly observed in a pH of range 3.8 to 4.5. In experiments where 0 to 6 M urea gradients were used, similar results were obtained (D. Graves, unpublished results). To determine which of the previously identified proteins these peaks represented, material in the two major peaks was further analyzed by SDS-PAGE and immunodiffusion by using monospecific antisera prepared against rechromatographed proteins as described later (Fig. 7). Protein from isoelectric focusing peaks of pH 5.6 (fraction no. 25, Fig. 6A) migrated in a region identical with p30 in SDS-PAGE (Fig. 6B). In immunodiffusion analysis, this material reacted with only antisera against p30, (see insert, Fig. 6A). These

FIG. 6. Isoelectric focusing and SDS-PAGE analysis of proteins from Tween 80-ether disrupted FeLV. (A) A sample of disrupted ^{14}C -amino-acid-labeled virus 10^6 counts/min; 2 mg of viral protein) was extensively dialyzed against 0.001 M Tris buffer, pH 7.4, digested with RNase (0.2 mg/ml) at 37 C for 20 min, clarified by centrifugation at 50,000 rpm, and then focused in a linear gradient of sucrose containing 2% Ampholine with a pH range of 3 to 10. Isoelectric focusing, collection of fractions, and assay of fractions for radioactivity, pH, and antigens by immunodiffusion were performed as described in Materials and Methods. Numbers 7 to 12 and 19 to 27 in the insert are isoelectric focusing fractions that were assayed with antisera against p15 (a) and p30 (b), respectively. (B and C) Proteins from the center of each major peak (fractions 9 and 25, respectively, from above) were dialyzed and concentrated by negative pressure dialysis in spacer gel buffer, dissociated, and electrophoresed as in Fig. 5. Fractionation of gels and assay of the fractions for radioactivity are as described in Materials and Methods. The positions of the Coomassie blue-stained FeLV protein bands are measured accurately from a gel run in parallel and drawn to scale at the top of part B for reference. Migration is from left to right.

FIG. 7. Immunodiffusion analysis of the FeLV proteins isolated by gel filtration in 6 M GuHCl. Outer wells contained proteins p30, p15, p11, p10, and p21, that had been rechromatographed two times (Fig. 3 and 4) and dialyzed to remove GuHCl. Center well A, antisera against Tween 80-ether disrupted virus. Center wells B, C, D, E, and F, antisera prepared against rechromatographed proteins p30, p15, p11, p10, and p21, respectively, after dialysis. A schematic drawing of the precipitin lines that developed is shown in the lower half of the figure.

data indicate that the protein in fraction no. 25 which focused at a pH of 5.6 was p30. Protein from isoelectric focusing peaks of pH 8.7 (fraction no. 9, Fig. 6A) migrated in a region

identical with p15 in 10% polyacrylamide gels (Fig. 6C). In immunodiffusion, this same material reacted only with antiserum against p15 (see insert, Fig. 6A). These data indicate that

the protein in fraction no. 9 which focused at a pH of 8.7 was p15.

The isoelectric points (pIs) for rechromatographed p30 and p15 were 5.5 and 8.9, respectively, which is in very good agreement with values obtained with Tween 80-ether-treated virus in the absence of GuHCl. The preliminary estimates of pIs for rechromatographed p11 and p10 were 4.2 and 3.8, respectively (Table 1). The pI for p10 was lower than expected since an equivalent avian protein appears to be basic (7) (D. Bolognesi, personal communication), and FeLV p10 has a higher ratio of arginine compared to other amino acids (D. Graves, unpublished data). Comparison with p11 and p10 from Tween-ether-disrupted virus was not done because they represent a small percentage of the virion (Table 1) and have not focused as distinct peaks (Fig. 6A). However, if the pIs are similar for the same polypeptides from the two different sources, as was the case for p30 and p15, the broad minor peak at pH 3.8 to 4.5 (Fig. 6A) may contain p11 and p10. This would have to be determined with a narrower pH range when larger amounts of radioactive virus became available.

Sucrose gradient sedimentation analysis of rechromatographed polypeptides. To obtain additional information about the relative size of isolated viral polypeptides, they were analyzed in linear gradients of 5 to 20% (wt/vol) sucrose. Proteins isolated from agarose columns tend to aggregate after removal of GuHCl and may exhibit false sedimentation values. To minimize this problem, the proteins were treated with 1 M GuHCl and then analyzed on a sucrose gradient containing 1 M GuHCl. Protein p30 was centrifuged in the same gradient with the 4.4S iodinated BSA, and p15, p11, and p10 were centrifuged in separate gradients with 2.54S iodinated chymotrypsinogen so that direct estimations of sedimentation values could be made. P30 sedimented at 2.5S; p15, 1.4S; p11, 1.17S; and p10, 1.14S (Table 1). P21 was not analyzed because insufficient amounts were available.

Immunological analysis of rechromatographed polypeptides renatured from guanidine hydrochloride. Gel filtration in GuHCl was not only suitable for preparative fractionation of viral proteins but allowed subsequent recovery of native antigenic and enzymatic activity of several of the proteins after dialysis (7, 23, 46). The isolated viral proteins were dialyzed free of GuHCl and the reducing agent and were tested in immunodiffusion with antisera produced against either Tween 80-ether-disrupted virus or against the rechromatographed renatured individual proteins. Antisera against Tween 80-ether-disrupted virus formed

precipitin lines only with p30, p15, and p11 (Fig. 7A). The single lines obtained with each protein cross one another indicating nonidentity between them. The nonidentity reaction between p15 and p11 is not shown in this figure (D. Graves and L. Velicer, unpublished observation). Antisera produced against rechromatographed proteins p30, p15, and p11 reacted only with their homologous proteins (Fig. 7B, C, and D, respectively). Single precipitin lines were obtained suggesting homogeneity. Antibodies were not detected against p10 and p21 with either antiserum against Tween 80-ether-treated virus or antiserum against individual proteins (Fig. 7A, E, and F).

Evidence of interspecies antigenic activity on the major FeLV polypeptide after gel chromatography in GuHCl. To determine if the interspecies antigenic determinant could be detected after gel chromatography in the presence of GuHCl, antisera prepared against p30 of the Rauscher MuLV (MuLV p30) was reacted with isolated FeLV p30, p15, and p11 in immunodiffusion along with the specific antiserum for each protein. The two precipitin bands formed between p30 and the two sera, anti-MuLV p30 and anti-FeLV p30, formed a line of identity further illustrating that the gs-3 antigenic activity was associated with the major polypeptide of the FeLV (Fig. 8). Furthermore, recovery of this antigenic determinant was still possible after the FeLV protein was chromatographed three times by GuHCl-agarose with subsequent removal of the GuHCl.

DISCUSSION

Analysis of Rickard's FeLV on 6% agarose in GuHCl resolved the six major proteins characteristic of the mammalian oncornaviruses (23). In addition, we report the identification of a minor component (p21) (Fig. 1A; J. Hoekstra, personal communication), whose existence was clearly verified by its iodination, isolation (Fig. 4E and F), and migration on SDS-PAGE (Fig. 5E). P21 may be similar to the seventh protein (19,000 daltons) from the avian RNA tumor viruses (7) that was reported missing in the mammalian RNA tumor viruses (23). If they are equivalent these data suggest that all C-type viruses may have the same number of small proteins but the amount of each may vary with the species as was already found for the four small polypeptides of several mammalian viruses (23). However, it is possible that p21 is a cell contaminant with a fortuitous molecular weight and further analysis is needed to rule this out.

Separation of glucosamine-labeled polypep-

FIG. 8. Immunodiffusion analysis of rechromatographed proteins p30, p15, and p11 with antisera prepared against MuLV gs (interspecies) antigen. Wells A, B, and C, antisera prepared against rechromatographed proteins p30, p15, and p11, respectively (Fig. 7). Well D, antisera prepared against rechromatographed protein p30 from Rauscher MuLV (MuLV p30) (gift from Erwin Fleissner and William Hardy, Sloan Kettering Institute, N. Y). Wells p30, p15, p11, proteins that had been rechromatographed twice on agarose columns (Fig. 3 and 4). A schematic drawing of the precipitin lines that developed is shown on the right half of the figure.

tides into two symmetrical peaks (7, 23; Fig. 1A) resulted in the concept of two glycoproteins among the six or seven major mammalian or avian proteins, respectively. However, in this study the data clearly show that FeLV gp ≥ 100 and gp70 are heterogeneous mixtures of several polypeptides (Fig. 2C, Table 1) despite being rechromatographed as symmetrical peaks on 6% agarose (Fig. 2A and B). The data for gp70 indicate the usefulness of 6% agarose for separating oncornavirus polypeptides is limited to proteins well below 60,000 daltons, despite a report of useful molecular weight estimations up to 80,000 (6). Even 4% agarose failed to adequately resolve gp70 (Fig. 1B) although the slightly better separation of ^{14}C amino acids suggests that several components may not be glycoproteins. It is also realized that some gp70 polypeptides may be cell contaminants. Further analysis with larger amounts of glucosamine-labeled virus will be needed to clarify these points and to correlate the six identifiable polypeptides of gp70, or possible unresolved minority species in the same peak, with the interspec II polypeptide(s) (44) or the RNA-directed DNA polymerase (46).

Analysis on 4% agarose revealed that gp ≥ 100 is actually an aggregate with an undetermined molecular weight of $\geq 220,000$ (Fig. 1B), based on the exclusion limit in the presence of 6 M GuHCl (45). However, since our basic preparative procedure involves 6% agarose the original identification is retained to define the source of

glycoprotein. Preliminary SDS-PAGE analysis of rechromatographed gp ≥ 100 showed that it dissociated to 100,000- to 115,000-dalton polypeptides (Table 1) in contrast to the largest avian glycoprotein (ml) which dissociated to a 32,000-dalton polypeptide (7). However, the absence of a smaller glycopolypeptide in gp ≥ 100 must be confirmed with labeled glucosamine because glycoproteins with a high carbohydrate content may have stained poorly with Coomassie blue and were not detected.

SDS-PAGE analysis of the small rechromatographed FeLV polypeptides clarifies the relative position of electrophoretic migration (Fig. 5) of the small mammalian polypeptides in comparison with their position of elution from agarose columns (Fig. 1A). Nowinski et al. (23) suggested that mammalian protein p2 (analogous to FeLV p15) migrates ahead of p3 (analogous to FeLV p11) and comigrates with p4 (analogous to FeLV p10) upon SDS-PAGE analysis. Under conditions of our study, FeLV p15 did not migrate ahead of FeLV p11. Instead, FeLV p11 and p10 comigrate (Fig. 5) and this superposition accounts for finding one less FeLV protein by SDS-PAGE than is found by gel filtration (23). Furthermore, these data indicate that several FeLV polypeptides migrate slightly differently from those of MuLV since Friend MuLV p15 and p12 form a broad band under similar conditions (D. Bolognesi, personal communication). Our data suggest that it may not always be possible to extrapolate for all mam-

malian viruses based on analysis of only one agent. The small variation in the molecular weights of p30, p21, p11, and p10 determined by the two methods (Table 1) may be due to differences in binding of SDS (33). Another possibility is that one or more of the small polypeptides contained lipid, carbohydrate, or glycolipids (5, 36, 40; D. Bolognesi, personal communication) which may affect migration in SDS-PAGE. The small FeLV polypeptides appear free of radioactive glucosamine (Fig. 1A), fucose, and galactose (L. Velicer, unpublished data), but trace amounts may not be detected by isotopic labeling methods.

In this study two innovations were used which extend the usefulness and effectiveness of the GuHCl-agarose method beyond that reported by other workers. The small scale column facilitates analytical work because it achieves equivalent or better separation, reduces operating costs five- to sixfold, and allows easy assay of the entire small fraction when radioactivity is limited (Fig. 1B; L. Velicer, unpublished data). The use of 8% agarose improves separation of the small polypeptides (Fig. 3 and 4), especially of p11 and p10, which presumably makes the molecular weight estimates more precise. These data indicate that 8% agarose is the gel of choice for optimal separation of polypeptides the size of p30 or smaller. Proof of complete separation includes the single peaks seen on SDS-PAGE (Fig. 6) with the occasional exception of a larger peak with p30 and p15. However, the larger peaks were not consistently observed when the same preparations were reanalyzed several times, suggesting aggregation of the monomeric polypeptide. P30 and especially p15 aggregate very rapidly after removal of GuHCl, and the conditions used for SDS-PAGE may have not achieved complete dissociation each time. Further proof that p30 and p15, and also p11, are completely separated is the lack of cross-reactivity in immunodiffusion analysis (Fig. 7). An entirely different concern is that small amounts of other contaminating protein(s) of identical size might be present since separation is only by molecular weight. Antisera to p30, p15, and p11 do not react with normal feline embryo fibroblast cell proteins in immunodiffusion analysis (L. Velicer, unpublished data) but this does not rule out low level or nonantigenic contaminants. Consequently, further purification by methods other than elution by molecular weight (D. Bolognesi, personal communication) may be needed to unequivocally prove homogeneity and permit further characterization by methods of protein chemistry (24, 26).

In this study an effort was made to determine pIs of the small polypeptides renatured from GuHCl and to compare them with results obtained with Tween-ether disrupted virus. The pIs determined for p30 and p15 from the two sources are in good agreement (Table 1). However, the values of 5.5 and 5.6 obtained for p30 from Rickard's FeLV contrast with a pI of 8.3 for the same polypeptide of the Theilen and Gardner strains of FeLV (11, 30, 41), despite our use of an identical isoelectric focusing method which included RNase treatment. Although RNA may be associated with our rechromatographed p30 and lower its pI, the ratio of optical density at 260 and 280 nm suggests less than 0.5% RNA before RNase treatment (L. Velicer, unpublished data). It is possible that these major proteins with different pIs have very different contents of several key amino acids but this would be unexpected since they each have the group-specific (4) and interspecies (11, 30, 31) antigenic determinants and presumably have the same function or structural role within the virion. Direct comparison of p30 from two different strains of FeLV under identical conditions is planned to resolve this question.

Removal of GuHCl permitted recovery of antigenic activity of three FeLV polypeptides (Fig. 7) including the gs-3 (interspecies) antigen (Fig. 8). The latter observation clarifies an important point left uncertain by a report that gs-3 activity of the major MuLV polypeptide could not be detected after GuHCl (23). The reaction of renatured FeLV p30 with antisera against renatured MuLV p30 clearly indicates that gs-3 activity on p30 of both species can be recovered after GuHCl treatment, and suggests that the negative results (23) were probably due to insufficient amounts of material. The antisera to FeLV p30, p15, and p11 are noncross-reacting, suggesting that they are immunologically unrelated as determined by immunodiffusion. The lack of immunological activity with FeLV p10 is reminiscent of earlier results with the 10,000-dalton avian (7) and murine (23) polypeptides. The negative results were first attributed to the small size (7) but it is now known that MuLV and FeLV p10 are immunologically reactive when sufficient protein is available for immunization (D. Bolognesi, personal communication).

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