JOURNAL OF VIROLOGY, Nov. 1971, p. 778-785
Vol. 8, No. 5

Chromatographic Separation and Antigenic Analysis of Proteins of the Oncornaviruses

I. Avian Leukemia-Sarcoma Viruses

ERWIN FLEISSNER

Division of Virology, Sloan-Kettering Institute, New York, New York 10021

Received for publication 18 August 1971

Gel filtration of avian tumor virus proteins in 6 M guanidine hydrochloride clearly resolved seven major protein species. The antigenic activity of these proteins was recovered in good yield after removal of the denaturing solvent, permitting a correlation of specific polypeptides with the principal antigens of the virion. Two of the proteins, of molecular weights 70,000 and 32,000, contain carbohydrate and are situated on the viral membrane, as shown by their being accessible in the intact virus to specific antibodies. Four proteins, with molecular weights (in guanidine) of 27,000, 19,000, 15,000, and 12,000, have different group-specific (gs) antigens and are enclosed within the viral membrane. The smallest protein, with a molecular weight of 10,000, has not previously been described; it is not detectable with antisera and possesses a mobility identical to that of one gs protein when subjected to electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate. Of the proteins lacking carbohydrate, three are present in the virion in a molecular ratio of 2:2:1, and the two others, present in almost equal amount, are rich in lysine and arginine.

Several studies have described proteins and antigens of the oncogenic ribonucleic acid (RNA) viruses, or oncornaviruses (18), of avian origin (1, 3, 4, 9, 10, 14). These studies agree in identifying glycoproteins in the virion with type-specific antigens and nonglycoproteins with group-specific (gs) antigens. There exists some disagreement, however, on the numbers of proteins and antigens in each class. The method of protein separation most generally used, polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS), offers good resolution, but the recovery of the original antigenic activities is not high since viral proteins are difficult to renature after exposure to SDS.

In the work to be reported here, a different method has been used for the analysis of viral polypeptides and antigens: gel filtration in the presence of 6 M guanidine hydrochloride and a reducing agent, as described by Tanford and his collaborators (11). Seven structural proteins are well resolved by this technique, and the relative molecular ratios of various proteins in the virion can be estimated. The resolution obtained in the low-molecular-weight range allows the identification of a small protein not previously described. Dialysis of particular protein fractions permits recovery of antigenic activity in high yield and the identification of specific polypeptides with the principal antigens of the virion. A subsequent report (Nowinski et al., *in preparation*) will extend this type of analysis to the mammalian oncornaviruses.

MATERIALS AND METHODS

Cells and viruses. Primary chick embryo cultures were prepared as described by Rubin (22) from 10-day-old COFAL-negative eggs (SPAFAS, Norwich, Conn.). The preparations of the Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV) and of unlabeled avian myeloblastosis virus (AMV) used here have been previously described (12). The Bryan high-titer strain of RSV, RAV-1 pseudotype, was obtained from H. Hanafusa, and avian leukosis virus, strain MC29, was from J. W. Beard.

Radioactive virus. Primary chick embryo cell monolayers were infected with avian leukosis virus, strain MC29, or AMV by the procedure of Langlois et al. (16). The appropriate labeled precursor was added to infected cells, in medium lacking Tryptose phosphate broth, for a period of 16 hr between the 3rd and 4th days after infection. ^{3}H-D-glucosamine (2.5 mCi/μmole, New England Nuclear Corp.) was added at 2 μCi/ml; ^{3}H-amino acid mixture (Schwarz BioResearch Inc.) was added at 10 μCi/ml. ^{3}H-L-arginine (750 μCi/μmole) and ^{3}H-L-lysine (2.8 mCi/μmole)

were obtained from New England Nuclear Corp., and ^{35}S-L-methionine (15 mCi/μmole) was from Amersham-Searle. These were added to culture media at levels of 10 μCi/ml.

Purification of radioactive virus. Culture fluid containing labeled virus was centrifuged for 10 min at 1,500 rev/min, and the supernatant fluid, except for the fraction immediately above the pellet of cell debris, was layered over a gradient of 15 to 60% sucrose in TNE buffer [0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, 0.1 M NaCl, 0.001 M ethylenediaminetetraacetate (EDTA), *p*H 7.4]. After centrifugation for 3 hr at 25,000 rev/min (SW27 rotor) fractions were collected from the bottom, and the virus band (density 1.16 to 1.18 g/cc) was diluted in TNE and pelleted by 1 hr of centrifugation at 25,000 rev/min.

Gel filtration of viral proteins in GuHCl. The virus pellet was dissolved in 0.5 to 1.0 ml of 8 M guanidine hydrochloride (GuHCl; Heico, Inc., Delaware Water Gap, Pa.) containing 2% mercaptoethanol, 0.01 M EDTA, and 0.05 M Tris-hydrochloride (*p*H 8.5) at 56 C for 45 min. Solid sucrose was then added to 10%, and chromatography was performed by the method of Fish et al. (11) on a column (82 by 1.6 cm) of Bio-Gel A-5m equilibrated with 6 M GuHCl, 0.01 M dithiothreitol (DTT), and 0.02 M sodium phosphate (*p*H 6.5). Samples of 50 μliters from the effluent fractions were mixed with 2 ml of water and counted in a scintillation counter after addition of a toluene-Triton X-100 (2:1) scintillation mixture (Packard Instruments). For complement fixation (CF) tests, fractions were dialyzed with four changes in the cold against 2.5 mM Tris-hydrochloride (*p*H 7.4). For other immunological tests, the dialyzed fractions were lyophilized, and the residues were dissolved in 0.1 or 0.05 volume of water.

PAGE. The procedure was that of Summers et al. (26), with 7.5% gels. In some experiments, gels containing 1% instead of 0.1% SDS were used with comparable results. Virus pellets or lyophilized fractions from gel filtration in GuHCl were dissolved in 50 μliters of solvent, containing 8 M urea (Mann Fine Chemicals, ultra pure), 5% mercaptoethanol, 1.5% SDS, and 0.025 M Tris-hydrochloride (*p*H 8.5), by heating for 2 min to 100 C. The samples were then applied directly to 7 by 0.5 cm gels. After electrophoresis for 16 hr at 2.5 v/cm, the gels were frozen and thawed and sliced into 1-mm slices. The slices were dissolved, singly or in pairs, in 0.5 ml of 30% H_2O_2 at 70 C for 6 hr and then counted in a dioxane-based scintillation cocktail.

Immunological techniques. The preparations of hamster COFAL sera (23) and rabbit hyperimmune serum (prepared against Tween-ether-split AMV) were those used previously (12). Rat sera against purified AMV antigens and rabbit serum against purified rat gamma globulin were obtained from R. Nowinski.

The procedures used for CF and Ouchterlony tests have been described (12).

Immunoelectrophoresis of AMV antigens, kindly carried out by R. Nowinski, was performed in 1% agar in barbital buffer (ionic strength 0.05) at *p*H 8.6.

Precipitation of ^{3}H-amino acid-labeled AMV was carried out as follows. To a 1-ml portion of freshly banded, diluted virus was added 0.25 ml of an appropriate rat serum prepared against a purified protein fraction from AMV. After incubation for 5 min at room temperature and 45 min at 4 C, 1.25 ml of rabbit anti-rat gamma globulin was added, and the incubation was repeated. The preparations were diluted with 2.5 ml of TNE buffer and centrifuged for 10 min at 5,000 rev/min in a Sorvall centrifuge. The supernatant fluids were layered onto 8-ml gradients of 15 to 60% sucrose in TNE and centrifuged for 1 hr at 36,000 rev/min in a Spinco SW41 rotor. Fractions of 0.5 ml were collected, and the total radioactivity at the density of virus was determined.

RESULTS

Comparison of viral proteins by gel filtration in guanidine hydrochloride and by electrophoresis in polyacrylamide gels. Strain MC29 of avian leukosis virus was chosen for many of the analyses employing amino acid-labeled virus because this strain grows to high titers in tissue culture and can be readily assayed (16). Purified virus labeled with a mixture of ^{3}H-amino acids was disrupted in 8 M GuHCl and 2% mercaptoethanol and subjected to chromatography on agarose gel in the presence of 6 M GuHCl and 0.01 M DDT. In the top panel of Fig. 1 is shown the elution profile of amino acid-labeled MC29 polypeptides and in the lower panel is shown the pattern obtained when ^{3}H-glucosamine was the labeled precursor. The volumes at which proteins elute in this system are proportional to the logarithms of their molecular weights. There are seven well separated peaks of radioactive protein, only two of which represent glycoproteins. These glycoproteins in the first two protein peaks in order of elution have been designated m1 and m2 (m = membrane) because they are found on the viral membrane (*see below*). Protein m1 elutes near the void volume of the column and has a molecular weight of $\geq$100,000; protein m2 has a molecular weight of 70,000. The next four peaks (gs1 to gs4) represent proteins with gs antigen activity (Fig. 2) and molecular weights in GuHCl of 27,000, 19,000, 15,000, and 12,000, respectively. The fifth of these late-eluting, nonglycoproteins, of molecular weight 10,000, is provisionally designated p5 since it is not known as yet whether it too is group-specific.

Gel filtration in GuHCl is not only suitable for preparative fractionation of viral proteins; subsequent dialysis allows recovery in good yield of native antigenic activity. CF titers as high as 256 per μg of protein have been obtained, which exceed by at least an order of magnitude those obtained after exposure of virus to SDS-containing buffers (10).

In Fig. 2 are compared the chromatographic profiles of unlabeled AMV proteins, represented by optical density at 280 nm (upper panel), and

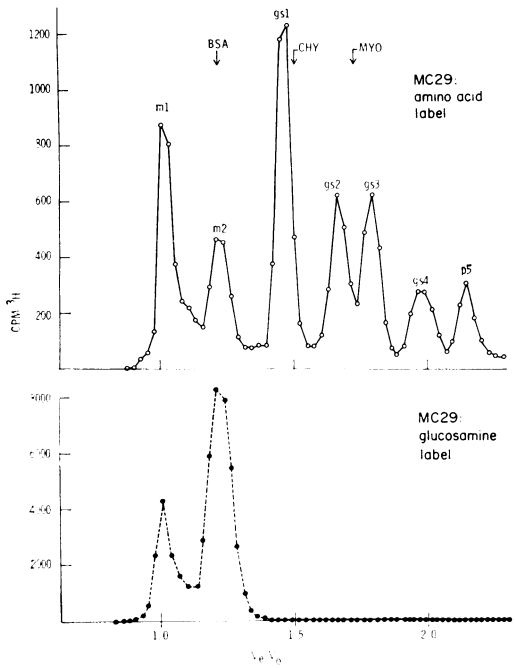

FIG. 1. *Gel filtration of MC29 viral proteins in the presence of 6 M GuHCl and 0.01 M DTT. Upper panel: virus labeled with a mixture of ³H-amino acids. Lower panel: virus labeled with ³H-glucosamine. To compute Ve/Vo, all fractions were individually weighed (cf. 11). The elution positions of three marker proteins are indicated: bovine serum albumin (molecular weight 69,000), chymotrypsinogen (molecular weight 25,700), and myoglobin (molecular weight 17,600).*

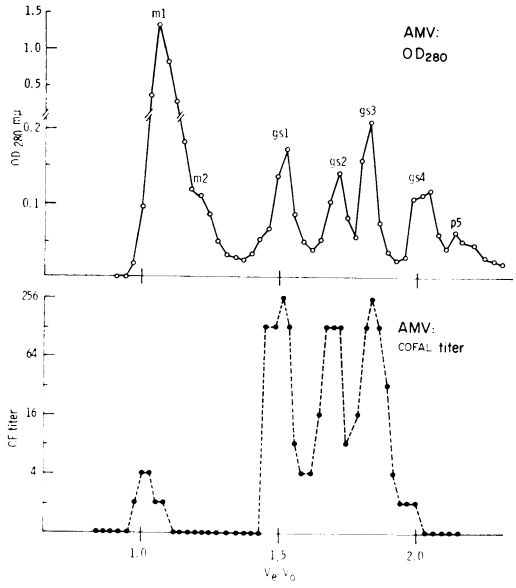

FIG. 2. *Gel filtration of AMV viral proteins in the presence of GuHCl and DTT and assay of gs antigen activity. Upper panel: optical densities at 280 nm, determined after dialyzing fractions individually to avoid the absorbance due to oxidized DTT. Lower panel: profile of CF activity obtained after gel filtration of a virus sample one-third as large as that used for the experiment in the upper panel. Hamster COFAL serum was used for CF assays.*

the pattern of CF activity after renaturation of individual protein fractions (lower panel). The serum used for CF was hamster COFAL serum, which is diagnostic for gs antigens (15). The same seven species of protein seen in Fig. 1 as peaks of radioactivity appear in Fig. 2 as peaks of optical density. Antigens reactive in CF were found at the positions of m1, gs1, gs2, gs3, and gs4. The activity associated with gs4 was small but reproducible and could be confirmed by Ouchterlony and immunoelectrophoretic analysis (*see* Fig. 6) after concentration of the antigen. In immunodiffusion tests, antigen in the m1 region reactive with COFAL serum formed a line of identity with gs1; aggregates of gs protein in the m1 fraction were also found in PAGE with radioactive preparations of this fraction (cf. Fig. 3). Thus, only the four gs proteins are in fact reactive with hamster COFAL serum. The high optical density near the void volume of Fig. 2 (upper panel) was due in part to light scattering since these fractions were visibly turbid, presumably due to membrane fragments from virus. Some of this excess optical density can also be ascribed to aggregation of gs proteins, which is more noticeable when amounts of virus representing considerable optical densities are chromatographed than when virus is used in tracer amounts.

A comparative study was next made of the behavior of viral proteins in GuHCl-gel filtration and in PAGE. In the upper two panels of Fig. 3 are presented the profile of viral proteins labeled with a radioactive amino acid mixture and the profile obtained when only ³H-glucosamine was used to label virus. Comparison with Fig. 1 shows that the relative positions of the major and minor peaks of glucosamine label were reversed in PAGE as compared to GuHCl chromatography. The PAGE profiles of individual GuHCl column fractions in the lower half of Fig. 3 confirm that in PAGE m1 travels as a smaller protein than m2. Molecular weight measurements show that the molecular weight of m2 is the same (70,000) in GuHCl and in PAGE (Table 1); m1, on the other hand, is an aggregate structure in GuHCl, which is dissociated to a monomer (molecular weight 32,000) in PAGE. Also, at the elution position of m1 in gel filtration and dissociating in PAGE were proteins with the mobilities of gs1 and gs2 as

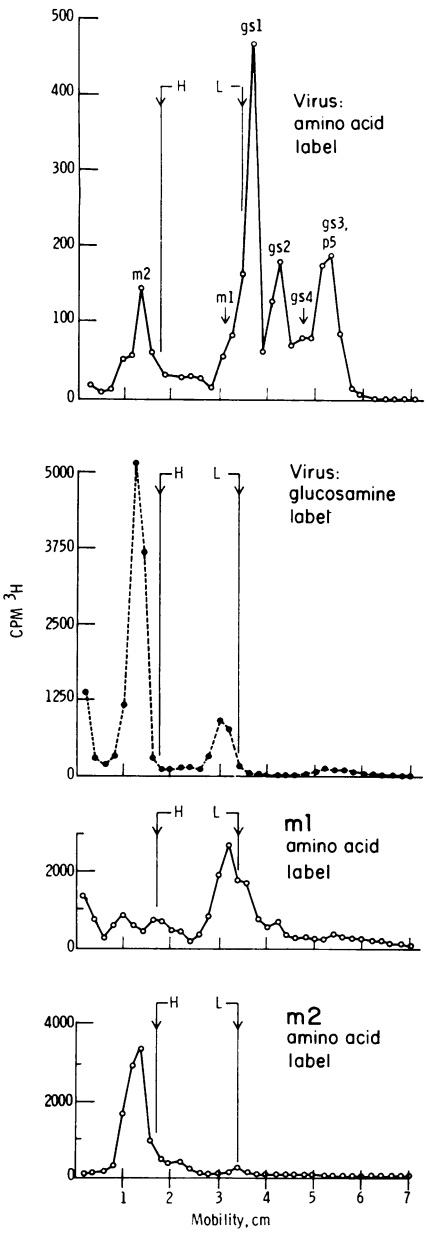

FIG. 3. *Polyacrylamide gel electrophoresis of MC29 viral proteins in the presence of SDS. The top two panels represent the profiles of proteins (³H-amino acid label) or glycoproteins (³H-glucosamine label) from virus preparations similar to those in Fig. 1. In the bottom two panels the materials used for electrophoresis were dialyzed and concentrated peak materials (ml and m2) from gel filtration of ³H-amino acid-labeled MC29 in GuHCl and DTT (cf. Fig. 1). The mobilities of the H and L chains of human gamma globulin are indicated.*

well as species (mobilities 1 to 2 cm) which were probably residual aggregates of ml.

When the four gs proteins and p5 isolated by GuHCl chromatography were likewise subjected to PAGE, the results in Fig. 4 were obtained. These proteins had the expected mobilities in PAGE, with the exception of gs3, which had an unexpectedly high mobility, equivalent to that of p5. Therefore, the fastest moving component in the analysis of proteins from whole virus in PAGE is actually composed of two proteins, gs3 and p5. The aggregation of ml in GuHCl chromatography and the superposition of gs3 and p5 in PAGE demonstrate the advantages of combining both techniques for the analysis of viral proteins.

Table 1 presents data on the molecular weights of the seven viral proteins, as obtained by the two methods. The aggregation of ml in GuHCl is reflected by its high apparent molecular weight in that system, as compared to PAGE. Reasonable agreement is found for the molecular weights of the other viral proteins in the two systems. The equivalence of apparent molecular weights for gs3 and p5 in PAGE is seen to result from an upward shift in the value for p5, as well as a shift downward in the value for gs3 in comparison to the values obtained in GuHCl. Both gs2 and gs4

TABLE 1. *Molecular weights of avian tumor virus proteins*[a]

Protein species	Mol wt determined by	
	GuHCl	PAGE
ml	100,000	32,000
m2	70,000	70,000
gs 1	27,000	25,500[b]
gs 2	19,000	21,000
gs 3	15,000	12,000
gs 4	12,000	14,000
p 5	10,000	12,000

[a] Molecular weights were determined by the method of Fish et al. (11) in guanidine hydrochloride (GuHCl) and that of Shapiro et al. (25) by polyacrylamide gel electrophoresis (PAGE). Markers used in GuHCl were bovine serum albumin, chymotrypsinogen, myoglobin, and the B and C chains of chymotrypsin. Markers in PAGE were bovine serum albumin, ovalbumin, human gamma globulin, H and L chains, chymotrypsinogen, and the B and C chains of chymotrypsin. In constructing calibration curves, the L chain of gamma globulin consistently displayed a mobility less than that of chymotrypsinogen (molecular weight 25,700).

[b] The gsl protein displayed a mobility in PAGE equal to that of chymotrypsinogen and slightly faster than the L chain of gamma globulin.

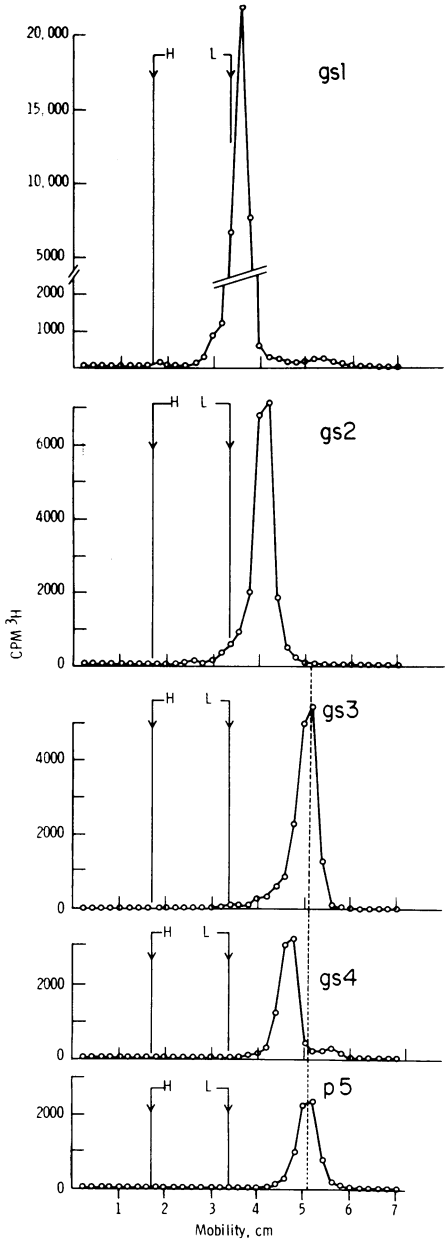

FIG. 4. *Polyacrylamide gel electrophoresis of MC29 viral proteins in SDS: proteins not containing carbohydrate. In each gel was used the indicated peak material from gel filtration of ³H-amino acid-labeled MC29 (cf. Fig. 1). Vertical lines show the positions of the H and L chains of human gamma globulin; the broken line in the last three panels indicates the equivalent mobilities of gs3 and p5.*

are somewhat retarded in PAGE, perhaps due to the influence of their high contents of basic amino acids (*see below*), even in the presence of SDS.

Ratios of protein molecules in the virion and their relative amino acid contents. Chromatography in GuHCl of MC29 labeled with a mixture of radioactive amino acids permitted a calculation of the relative numbers of protein molecules of each type present in the virion. The per cent of total radioactivity in each protein species was measured, and the values were divided by the molecular weights of the polypeptide chains. The results of this calculation are presented in Table 2. Two proteins, gs1 and gs3, are represented by the most copies in the virion and are present in equimolar amounts; p5 is present in half the number of copies of these two. These results suggest the possibility that these three proteins are united in a major structural element of the virion. The two other gs proteins, gs2 and gs4, are present in almost equal amounts in the virion. As will be shown below, gs2 and gs4 are both proteins rich in basic amino acids.

To characterize further the gs proteins and p5, their relative contents of three amino acids, methionine, lysine, and arginine, were computed from gel filtration profiles of virus labeled with individual amino acids (Table 3). To fix absolute values for amino acid contents, the data of Niall et al. for gs1 were used (see legend to Table 3). The values thus obtained for methionine content are consistent with the numbers of peptide fragments obtained by cyanogen bromide cleavage of

TABLE 2. *Molar ratios of proteins in MC29 virus and approximate molecular composition of the virion*

Protein species	Molar ratio[a]	Estimated no. of molecules[b]
m1	0.35[c]	1,000
m2	0.15	400
gs 1	1.00	3,000
gs 2	0.60	1,800
gs 3	1.02	3,000
gs 4	0.67	2,000
p 5	0.51	1,500

[a] Molar ratios were computed from radioactivity profiles of four separate gel filtration analyses of virus labeled with mixed radioactive amino acids, applying as a correction factor for each protein the molecular weights given in Table 1 [average of guanidine hydrochloride (GuHCl) filtration and polyacrylamide gel electrophoresis (PAGE) results] (8).

[b] Numbers of molecules per virion were calculated assuming a viral molecular weight of 4×10^8 and a protein composition of 65% (28).

[c] Amount of protein represented by m1 has been corrected for the presence of gs1 and gs2 in this area of the GuHCl filtration profile, as determined by PAGE analysis. No correction has been made for carbohydrate content (unknown) in m1 and m2.

TABLE 3. *Amino acid contents of MC29 viral proteins*[a]

Protein species	Calculated amino acid residues/molecule		
	Methionine	Lysine	Arginine
gs 1	5.0	8.0	12.0
gs 2	4.4	15.2	7.6
gs 3	3.6	4.3	7.4
gs 4	2.2	7.4	20.9
p 5	0.8	<0.3	8.9

[a] Amino acid residues per molecule of protein were computed from gel filtration profiles of virus labeled with individual radioactive amino acids by measuring the area under each peak and correcting for the relative number of copies of that protein molecule in the virion (Table 2). Integral values have been assigned for gs1, based on the data of Niall et al. (17), which then fix the values for each amino acid in the other proteins. Values for AMV reported by Niall et al. for gs1 (their gs a) are 4 met, 8 lys, and 12 arg; for gs3 (their gs b), they report 5 met, 4 lys, and 7 arg.

individual proteins, and the values for lysine content of gs2 match the number of lysine-labeled tryptic peptides obtained from this protein (*unpublished data*).

The data in Table 3 show that gs2 and gs4 are rich in lysine and arginine, respectively. About one-third of the amino acid residues in gs4 consist of lysine or arginine. Thus, it would appear likely that this protein is associated with the RNA in the virion. The amino acid contents of these five proteins demonstrate unequivocally that they are distinct polypeptides; in particular, data for gs3 and p5 establish that these proteins, despite their identical mobilities in PAGE, cannot be different conformational forms of the same protein.

Serological analysis of viral proteins renatured from guanidine hydrochloride. Representative Ouchterlony patterns for three gs proteins (gs1, gs2, and gs3) with hamster COFAL serum are shown in Fig. 5. The antigens used in these tests were AMV proteins purified by chromatography in GuHCl. All three proteins show reactions of nonidentity. The fourth gs protein, gs4, also shows nonidentity with the other three gs proteins (not shown in Fig. 5). In some platings of gs1, gs2, and gs3, secondary precipitin lines could be resolved (cf. gs3 in Fig. 5). Other workers have also described two precipitin lines associated with a single gs protein (gs1) in both avian and murine tumor viruses (3, 13). The significance of these secondary lines, which may be due to specific aggregates of gs proteins, is under study.

Additional serological analyses were carried out in collaboration with Robert Nowinski. The four gs proteins isolated from AMV by chromatography in GuHCl were found to yield characteristic, nonidentical precipitin lines with 15 different hamster COFAL antisera. The gs character of these proteins was confirmed when lines of identity were obtained by individually plating the four gs proteins from AMV next to ether-treated AMV (cf. Fig. 5), BH-RSV (RAV-1), MC29, and SR-RSV.

Immunoelectrophoresis of the gs proteins from AMV using a hyperimmune rabbit serum prepared against Tween-ether-split virus is depicted in Fig. 6. A unique arc of precipitation is observed for each protein, the most basic being gs1, with a mobility corresponding to that of mouse gamma globulin (pI = 8.6). Although gs4 displayed only weak CF activity in GuHCl chromatography (Fig. 2), upon concentration this protein displayed a strong, characteristic arc in immunoelectrophoresis.

Precipitation in agar was not observed with the p5 fraction of AMV with hamster sera, the hyperimmune rabbit serum, or rat serum prepared against p5 itself.

The precipitation from liquid medium of radiolabeled AMV by specific antisera was used to de-

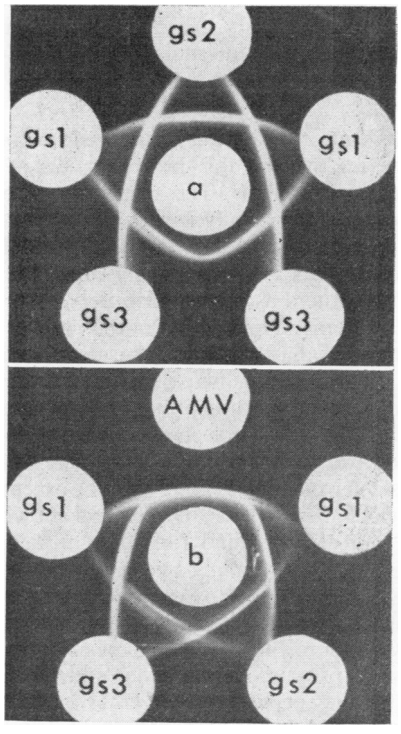

FIG. 5. *Ouchterlony analysis of AMV antigens isolated by gel filtration in GuHCl and DTT and subsequent dialysis. Outer wells contain ether-treated AMV or the indicated peak materials (cf. Fig. 2) from gel filtration. Center wells (a and b) contain hamster COFAL serum.*

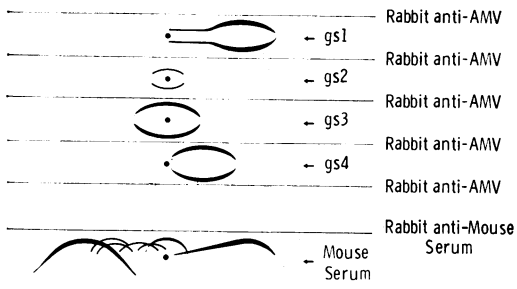

FIG. 6. *Immunoelectrophoresis of gs antigens from AMV, prepared by gel filtration in GuHCl and DTT and subsequent dialysis. The cathode is on the right. The pattern of mouse serum proteins is shown for comparison. The author is indebted to R. Nowinski for this analysis.*

termine which proteins of intact virus were accessible to antibody (*see above*). Treatment of virus with individual rat antisera prepared against m1 or m2 resulted in reductions in the banded virus of 80 and 21%, respectively, whereas no loss in banded virus was observed when antisera prepared against gs1, gs2, gs3, or gs4 were used. Thus both m1 and m2 appear to be located on the surface of the virion so as to be at least partially accessible to antibody. All of the gs proteins, on the other hand, are internal.

DISCUSSION

Gel filtration in 6 M guanidine hydrochloride is a powerful method for the separation of avian tumor virus proteins. The method may be employed for either analytical or preparative purposes; in the latter case removal of GuHCl by dialysis permits a good recovery of antigenic activity (CF titers as high as 256 per μg for some AMV proteins). Application of the method in the present work has permitted the identification of seven viral proteins, two of them glycoproteins and four of the remaining five possessing gs antigenic activity. The molecular weights of the proteins determined in GuHCl agree quite well with values determined in PAGE, with the exception of m1, which behaves as an aggregate in GuHCl. Small differences found for other proteins may derive from SDS-binding by viral proteins which differs from the standard value of 1.4 mg of SDS per mg of protein (19). Thus gs1 and gs3 may bind relatively more SDS, and p5 less SDS, than an "average" protein. Alternatively, some secondary structure may persist in either GuHCl or PAGE.

Protein p5 has not previously been reported as a constituent of these viruses because its mobility in PAGE is identical to that of gs3 and because it does not have antigenic activity, even against sera prepared with p5 itself as the immunizing antigen. This lack of antigenicity probably derives from the small size of the protein. This protein has invariably been found in chromatography of virus in GuHCl, regardless of the source or mode of purification of the virus sample. Additionally, Nowinski and collaborators have found a similar protein in preparations of RNA tumor viruses from three mammalian species (*in preparation*).

The results presented here for glycoproteins agree best with those of Duesberg et al. (9), who defined only two prominant glycoprotein components in the virion. In the present work the two glycoproteins, m1 and m2, were found to be on the exterior of the viral membrane in a configuration available to specific antibodies. Further evidence that these proteins both reside in the viral membrane is the isolation of these two proteins in a complex when virus was solubilized in mild detergent (9, 14). This complex of viral glycoproteins was found to absorb virus-neutralizing antibody (9), and the glycoproteins from different virus strains exhibited slightly different mobilities in PAGE, consistent with a role in the strain-specific antigens of the viral surface (9, 20). Studies by D. Rifkin and R. Compans (*personal communication*) show that, as with other lipid-coated viruses of the RNA type (5, 6, 8, 24), the glycoproteins of RSV are located on the exterior of the viral membrane and can be removed by mild proteolytic hydrolysis.

For the gs proteins the results presented here agree most closely with those of Bolognesi and Bauer (4), except that the component with the highest mobility in their PAGE gels actually comprises two proteins (gs3 and p5). These authors also found four gs antigens (3) with the same relative mobilities in PAGE as gs1–gs4. The proteins designated gs a and gs b by Allen et al. (1) correspond to gs1 and gs3, by their relative molecular weights and amino acid compositions (reference 17; Table 3). In general, where some losses of gs antigen activity have occurred, gs1 and gs3 are most likely to be detected; thus these two proteins constitute the strongest antigens when viral proteins are recovered from solvents containing 4 M urea (14) or SDS (10).

The data on relative contents of basic amino acids in the gs proteins (Table 3) correlate reasonably well with the relative charges of the native proteins at *p*H 8.6 (Fig. 6), given the absence at this time of data on contents of acidic amino acids. There is reasonable agreement also with the *p*I values reported by Hung et al. (14) if allowance is made for the fact that their values are reported for proteins in the presence of 4 M urea, and in conditions of low ionic strength in which residual protein-RNA interactions might occur. The latter effect could explain the low *p*I which these authors

report for gs4, which contains a high proportion of lysine and (especially) arginine for a small protein (Table 3).

Of gs proteins in their native configurations, gs1 and gs4 are the most basic (Fig. 6); however, there is evidence that the content of gs1 is much reduced in preparations of viral "cores" (21). Thus gs4 may well be one protein in direct association with viral RNA; gs1, perhaps in concert with gs3 and p5, could play a role analagous to that postulated for the major structural protein of influenza virus, that of providing an inner protein "shell" between the viral membrane and the viral ribonucleoprotein (7, 8). Consistent with this interpretation is the finding that in viral core preparations there is a dramatic enrichment of gs4 and a reduction in the amounts of gs1, gs3, and p5, compared with the amounts of these proteins in whole virus (21; *unpublished data*). More experiments are obviously needed to test these hypotheses, as well as to determine the relationship of these proteins to the other internal virus proteins, such as the viral reverse transcriptase (2, 27).

ACKNOWLEDGMENTS

I thank Ellen Tress for expert technical assistance. I am indebted to Peter Model for first suggesting the use of guanidine hydrochloride in the separation of viral proteins and to Peter Gomatos and other members of this division for helpful discussions.

This investigation was supported by Public Health Service grant CA 08758 from the National Cancer Institute and by the John A. Hartford Foundation, Inc.

LITERATURE CITED

1. Allen, D. W., P. S. Sarma, H. D. Niall, and R. Sauer. 1970. Isolation of a second group-specific antigen (gs-G) from avian myeloblastosis virus. Proc. Nat. Acad. Sci. U.S.A. 67:837–842.
2. Baltimore, D. 1970. RNA-dependent DNA polymerase in virions of RNA tumor viruses. Nature (London) 226:1209–1211.
3. Bauer, H., and D. P. Bolognesi. 1970. Polypeptides of avian RNA tumor viruses. II. Serological characterization. Virology 42:1113–1126.
4. Bolognesi, D. P., and H. Bauer. 1970. Polypeptides of avian RNA tumor viruses. I. Isolation and chemical analysis. Virology 42:1097–1112.
5. Chen, C., R. W. Compans, and P. W. Choppin. 1971. Parainfluenza virus surface projections: glycoproteins with haemagglutinin and neurominidase activities. J. Gen. Virol. 11:53–58.
6. Compans, R. W. 1971. Location of the glycoprotein in the membrane of Sindbis virus. Nature N. Biol. 229:114–116.
7. Compans, R. W., and N. J. Dimmock. 1969. An electron microscopic study of single-cycle infection of chick embryo fibroblasts by influenza virus. Virology 39:499–515.
8. Compans, R. W., H.-D. Klenk, L. A. Caliguiri, and P. W. Choppin. 1970. Influenza virus proteins. I. Analysis of polypeptides of the virion and identification of spike glycoproteins. Virology 42:880–889.
9. Duesberg, P. H., G. S. Martin, and P. K. Vogt. 1970. Glycoprotein components of avian and murine RNA tumor viruses. Virology 41:631–646.
10. Duesberg, P. H., H. L. Robinson, W. S. Robinson, R. J. Huebner, and H. C. Turner. 1968. Proteins of Rous sarcoma virus. Virology 36:73–86.
11. Fish, W. W., K. G. Mann, and C. Tanford. 1969. The estimation of polypeptide chain molecular weights by gel filtration in 6 M guanidine hydrochloride. J. Biol. Chem. 244:4989–4994.
12. Fleissner, E. 1970. Virus-specific antigens in hamster cells transformed by Rous sarcoma virus. J. Virol. 5:14–21.
13. Gilden, R. V., O. Oroszlan, and R. J. Huebner. 1971. Coexistence of intraspecies and interspecies specific antigenic determinants on the major structural polypeptide of mammalian C-type viruses. Nature N. Biol. 231:107–108.
14. Hung, P. P., H. L. Robinson, and W. S. Robinson. 1971. Isolation and characterization of proteins from Rous sarcoma virus. Virology 43:251–266.
15. Kelloff, G., and P. K. Vogt. 1966. Localization of avian tumor virus group-specific antigen in cell and virus. Virology 29: 377–384.
16. Langlois, A. J., D. P. Bolognesi, R. B. Fritz, and J. W. Beard. 1969. Strain MC29 avian leukosis virus release by chick embryo cells infected with the agent. Proc. Soc. Exp. Biol. Med. 131:138–143.
17. Niall, H. D., R. Sauer, and D. W. Allen. 1970. The N-terminal amino acid sequence of two avian leukosis group-specific antigens. Proc. Nat. Acad. Sci. U.S.A. 67:1804–1809.
18. Nowinski, R. C., L. J. Old, N. H. Sarkar, and D. H. Moore. 1970. Common properties of the oncogenic RNA viruses (oncornaviruses). Virology 42:1152–1157.
19. Reynolds, J. A., and C. Tanford. 1970. The gross conformation of protein-sodium dodecyl sulfate complexes. J. Biol. Chem. 245:5161–5165.
20. Robinson, W. S., P. Hung, H. L. Robinson, and D. D. Ralph. 1970. Proteins of avian tumor viruses with different coat antigens. J. Virol. 6:695–698.
21. Robinson, W. S., and H. L. Robinson. 1971. DNA polymerase in defective Rous sarcoma virus. Virology 44:457–462.
22. Rubin, H. 1960. An analysis of the assay of Rous sarcoma cells in vitro by the infective center technique. Virology 10:29–49.
23. Sarma, P. S., H. C. Turner, and R. J. Huebner. 1964. An avian leukosis group-specific complement-fixation reaction. Application for the detection and assay of non-cytopathogenic leukosis viruses. Virology 23:313–321.
24. Schulze, I. T. 1970. The structure of influenza virus. 1. The polypeptides of the virion. Virology 42:890–904.
25. Shapiro, A. L., E. Viñuela, and J. V. Maizel. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gels. Biochem. Biophys. Res. Commun. 28:815–820.
26. Summers, D. F., J. V. Maizel, Jr., and J. E. Darnell, Jr. 1965. Evidence for virus-specific noncapsid proteins in poliovirus-infected HeLa cells. Proc. Nat. Acad. Sci. U.S.A. 54:505–513.
27. Temin, H. M., and S. Mizutani. 1970. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature (London) 226:1211–1213.
28. Vogt, P. K. 1965. Avian tumor viruses, p. 293–385. *In* K. M. Smith and M. A. Lauffer (ed.), Advances in virus research. Academic Press Inc., New York.