STRUCTURAL POLYPEPTIDES OF PRIMATE DERIVED TYPE C RNA TUMOR VIRUSES

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Received November 20, 1973

SUMMARY

Proteins of gibbon ape lymphosarcoma virus (GaLV) and woolly monkey sarcoma virus, type 1, together with its associated virus (SSV-1/SSAV-1) were analyzed by guanidine-agarose chromatography and the separation patterns were compared with those of mouse and feline type C viruses. GaLV contained five major proteins, including two glycoproteins, whereas lower mammalian viruses contained six major proteins, including two glycoproteins. The molecular weights of the five GaLV proteins closely resembled the molecular weights of the five equivalent lower mammalian viral proteins. SSV-1/SSAV-1 showed a separation pattern similar to GaLV except it contained a low but detectable amount of an additional glycoprotein. Both GaLV and SSV-1/SSAV-1 were deficient in a protein of molecular weight about 15,000 daltons which is found in all known type C viruses of avian, reptilian and lower mammalian species.

INTRODUCTION

Two representative type C RNA tumor viruses directly isolated from primates are known, the woolly monkey sarcoma virus, type 1, together with its associated virus (SSV-1/SSAV-1) and gibbon ape lymphosarcoma virus (GaLV) (1-3). These viruses differ in their major internal polypeptide (group-specific antigen) and DNA

polymerase from all other known type C viruses (3-5). Here we describe the major polypeptide composition of GaLV and SSV-1/SSAV-1. It is noted with surprise that the polypeptide composition of these primate derived viruses differs significantly from the type C viruses from lower mammals, namely feline and mouse, in that they apparently lack a nonglycosylated protein of molecular weight about 15,000 daltons.

MATERIALS AND METHODS

Source of Materials. Radiochemicals, 6-^{3}H-D-glucosamine-hydrochloride (7.3 Ci/mmole) and ^{14}C-L-amino acids were obtained from New England Nuclear Corp.; molecular weight markers: bovine serum albumin, ovalbumin, chymotrypsinogen A, myoglobin, cytochrome C from Schwarz/Mann and phosphorylase a from Worthington; guanidine hydrochloride (Gu.HCl) from Sigma; agarose, acrylamide and bisacrylamide from Bio Rad Lab.; Eagle's minimum essential medium (MEM) and fetal bovine serum (FBS) from Flow Laboratories. Sodium dodecyl sulfate (SDS) obtained from Matheson was recrystalized prior to use.

Cells and Viruses. GaLV and SSV-1/SSAV-1 were isolated from human rhabdomyosarcoma cell cultures (RD) (6) in passage levels 99 and 104 after they had been infected at passage levels 91 and 100, respectively (McAllister, unpublished). A type C virus strain, 292, derived from a wild mouse, was isolated from an infected NIH Swiss mouse embryo cell culture (7). Gardner-Arnstein strain of feline leukemia virus, FeLV(GA), was isolated from RD cells infected with this virus (8). Cultures were maintained in MEM containing 10% FBS.

Radiolabeling of Viruses. The virus producing cells were grown in complete MEM plus 10% dialyzed FBS in presence of ^{14}C-

amino acid mixtures and ^{3}H-glucosamine, each at 2 μCi/ml. Two changes of the medium were made at 24 hr intervals. Viruses were purified from the pooled culture fluids (200 ml) by the usual procedure (9, 10).

Analysis of Viral Proteins. Virion proteins were analyzed by agarose (Biogel A-5m, 200-400 mesh) column chromatography in 6M Gu.HCl followed by SDS-polyacrylamide gel electrophoresis.

Gel filtration was carried out as described by Nowinski et al (11). ^{14}C-amino acid and ^{3}H-glucosamine labeled virus pellets were dissolved in 0.4 ml of a solution of 8M Gu.HCl, 0.01 M EDTA, 0.05 M Tris-HCl and 2% β-mercaptoethanol (pH 8.5) by heating at 56^{o} for 45 min. The solution was then applied on a column, 1.5 X 87 cm, of Biogel A-5m equilibrated with 6 M Gu.HCl in 0.02 M sodium phosphate, pH 6.5, and 0.01 M dithiothreitol. Elution was carried out with the same buffer and fractions of about 0.8 ml were collected. Aliquots (0.1 ml) of the fraction were counted differentially for ^{3}H and ^{14}C in a Beckman LS-250 liquid scintillation system (10). Peak radioactive fractions were pooled and dialyzed against 100-fold excess of 0.002 M Tris-HCl, pH 7.4, containing 0.1% β-mercaptoethanol at 5^{o} for 4 days with 6 changes. The dialyzed solutions were dried by lyophilization.

A modified procedure (10) of Summers et al (12) was used for electrophoresis of the peak materials on 7.5% polyacrylamide--0.2% bisacrylamide--0.1% SDS gels at 9 mAmp/gel for 4 hr at room temperature. After the run the gels were taken out of the tubes, frozen at -80^{o} and cut into 1 mm slices. Two slices were taken as one fraction and counted in 4 ml of scintillation fluid (8.0 g butyl PBD, 0.5 g PBBO in 750 ml toluene and 250 Triton X-100) after shaking with 0.4 ml of 2% periodic acid

for 30 min at 60°. Small glass vials (Cal-Glass) and standard size carrier glass vials were used. Standard proteins of known molecular weights were always included in identical gels. For staining, gels were fixed in 20% sulphosalisylic acid for 18 hr and stained in Coomassie brilliant blue (13).

RESULTS

Virion proteins and glycoproteins labeled with ^{14}C-amino acids and ^{3}H-glucosamine were separated by gel filtration in Gu.HCl agarose column. Fig 1 shows the distribution of radio-

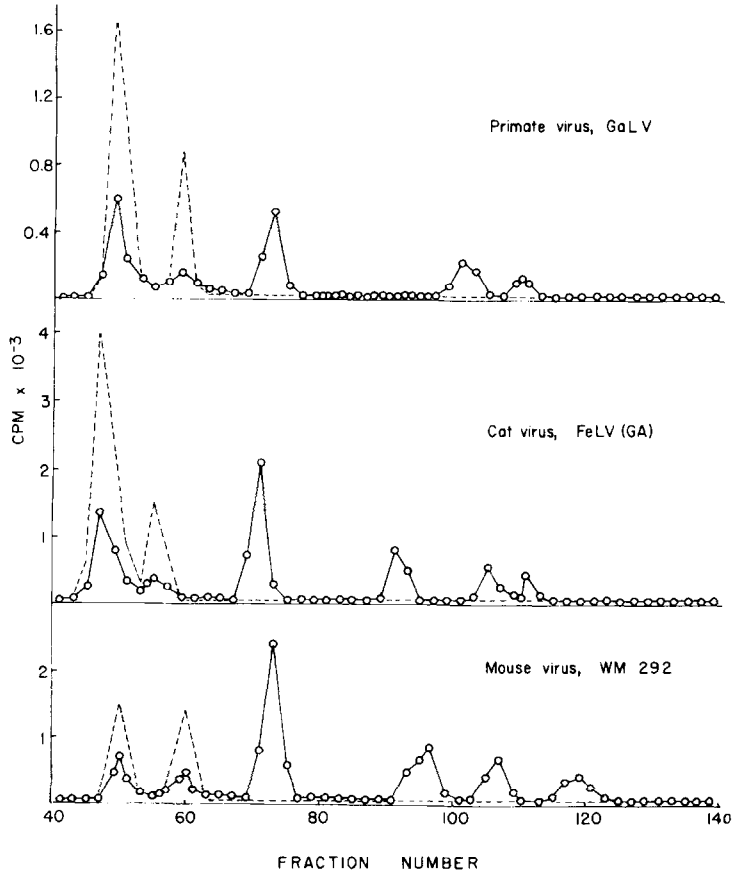

Fig. 1 Gel filtration patterns in Gu.HCl of mouse, cat and gibbon ape type C viruses simultaneously labeled with ^{3}H-glucosamine and ^{14}C-amino acid mixtures. - - - - ^{3}H; ——— ^{14}C.

activity into the major polypeptides of GaLV, FeLV(GA) and WM-292 virions. All of the peaks contained the ^{14}C-label but only the first two peaks (gp1 & gp2) contained the ^{3}H-label indicating that the first two components were viral glycoproteins. The separation of FeLV(GA) or WM-292 into six major peaks was consistent with the general protein composition of type C feline and mouse RNA tumor viruses (10, 11).

Fig 2 shows the separation pattern of SSV-1/SSAV-1 virion

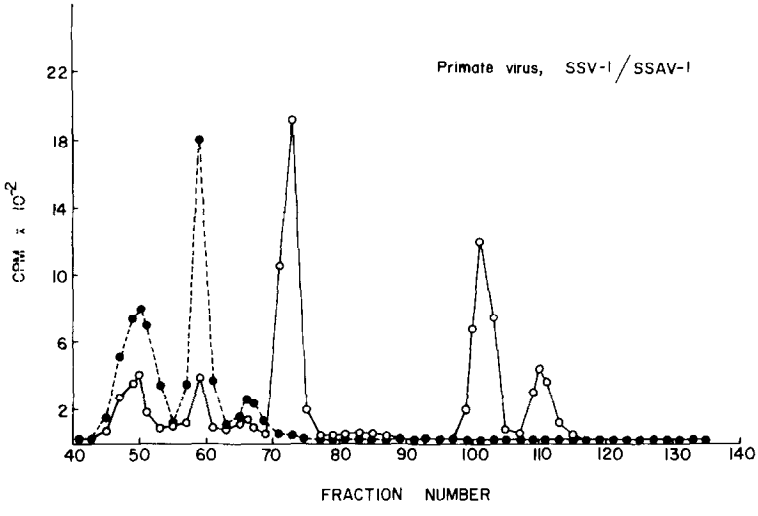

Fig. 2 Gel filtration pattern in Gu.HCl of woolly monkey type C virus (SSV-1/SSAV-1) simultaneously labeled with ^{3}H-glucosamine and ^{14}C-amino acid mixtures. - - - - ^{3}H; ——— ^{14}C.

polypeptides under identical conditions. It is similar to GaLV except it contained a low but detectable amount of an additional glycoprotein. At the time of writing this manuscript we became aware of another study showing that SSV-1/-SSAV-1 released from marmoset skin cells also contained three glycosylated and three non-glycosylated polypeptides (Hoekstra

and Deinhardt, personal communication). Figs 1 and 2 also show that a common structural feature of the primate derived viruses was that they were deficient in a non-glycosylated polypeptide present in lower mammalian viruses (fourth protein peak, from left, of WM-292 or FeLV).

The estimated molecular weights of the five structural protein groups of GaLV, based on their relative electrophoretic mobility in SDS-polyacrylamide gel, are summarized in Table 1.

Table 1. Approximate Molecular Weights of the Major Proteins of GaLV as Determined by SDS-Polyacrylamide gel Electrophoresis.

Proteins from guanidine-agarose chromatography*	Molecular weight (daltons)+
1 (gp 1)**	100,000
2 (gp 2)	70,000
3	29,000
4	11,500
5	10,000

* Peaks in decreasing order of molecular size.
\+ Average values from 2 to 4 determinations.
** Penetrance of gp1 into SDS-gels was partial, perhaps because of its association with lipids and glycolipids of the viral membrane.

These values corresponded closely to those of the five equivalent proteins of type C viruses derived from lower mammals (10, 11).

DISCUSSION

These results demonstrate that type C viruses isolated from subhuman primates are composed, with one exception, of

similar structural proteins as found in known type C mammalian RNA tumor viruses. A striking difference, however, is the absence or undetectable amount of a non-glycosylated polypeptide. This missing polypeptide has a molecular weight of 14,500 to 16,000 daltons in avian and lower mammalian species (10, 11). Thus, there are five major non-glycosylated polypeptides in avian type C viruses (11), four in mouse, hamster, feline and reptilian type C viruses (10, 11, 14), and three in the primate derived type C viruses reported here.

Thus, the protein pattern on guanidine-agarose chromatography may be summarized in categories depending on the species of virus isolation (Table 2). Since p2 and p4 of avian leukemia virus have similar tryptic peptides, it was suggested that p4 might be derived from the higher molecular weight protein p2 by cleavage (14). A relationship between p2 and p4 might resolve the difference between avian and rep-

Table 2. Guanidine-agarose Chromatography Patterns of Type C Virus Proteins

Species of Isolation	Proteins						
	gp1*	gp2*	p1	p2	p3	p4	p5
Avian	+	+	+	+	+	+	+
Reptilian or lower mammals (mouse, hamster, feline)	+	+	+	-	+	+	+
Primate (gibbon ape and woolly monkey)	+	+	+	-	-	+	+

+, present; -, absent or undetectable.
* Additional minor species of glycoproteins may exist in type C viruses (14). In the present study a third glycoprotein peak was detected only in SSV-1/SSAV-1. Finding of two prominent glycoprotein species, gp1 and gp2, is, however, generally agreed upon (10, 11, 14).

tilian or lower mammalian type C viruses in their content of proteins with independent primary structures. But now with the results of primate derived viruses, the issue becomes more complicated. Is the deficient protein a result of gene deletion, nonsense mutation or evolvement of better controls to exclude nonessential polypeptide(s) from the virion structure? It would be interesting to look into the structural proteins of a human derived type C virus, if it is ever released, or if it contains all gene functions required for its structural asembly.

ACKNOWLEDGEMENT

We thank Mss. Y. Yuan, J. Filbert and M. Peer for assistance. This research was conducted under contract No. PH-43-NCI-68-1030 within the Virus Cancer Program of the National Cancer Institute, National Institutes of Health, U.S. Public Health Service, Department of Health, Education, and Welfare.

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