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55,000-Dalton, Retrovirus-Associated, Cell Membrane Glycoprotein: Purification and Quantitative Measurements of Expression in Viruses, Cells, and Tissues

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We have purified to homogeneity and characterized a 55,000-dalton rat cell membrane glycoprotein, gp55. This protein was originally identified in preparations of a defective pseudotype of the Kirsten sarcoma virus and shown to be present in several rodent retrovirus particles. The gp55 was purified from this defective virus by concanavalin A and heparin affinity chromatography, as well as by preparative sodium dodecyl sulfate-gel electrophoresis. Both preparations displayed similar purity and antigenic characteristics. The ^{125}I-labeled gp55 was precipitated by antisera against rodent retroviruses, but not by monospecific antisera against purified type C virus structural proteins, thus indicating that gp55 was retrovirus associated, but unrelated to known retrovirus structural proteins. Competition radioimmunoassay with an anti-rat virus serum which recognized rodent group-specific antigens on gp55 indicated: (i) the presence of gp55 antigens in 15 rodent cell lines, but not 10 nonrodent cell lines; (ii) no effect of viral infection or cell transformation on the amount of gp55 expressed; (iii) up to 100-fold increases in the concentration of the gp55 antigens in nine rodent retroviruses, but not in five nonrodent viruses, as compared to cells; (iv) the presence of gp55 in rodent sera, especially of the NZB mouse, where anti-gp55 antibody was also detected; (v) a lymphoid and epithelial tissue distribution of gp55 in rats and mice. Additional competition radioimmunoassays with a broad-reacting antivirus serum also detected the presence of gp55 in nonrodent, mink, and human cells and thus distinguished rat type, rodent group, and interspecies antigenic determinants on gp55. In conclusion, gp55 is a cell membrane glycoprotein associated in high concentration with retroviruses.

The type C oncoviruses are enveloped ribonucleic acid (RNA) retroviruses which form particles by budding through the plasma membranes of infected cells (25). The virus encodes six structural proteins, of which two form the envelope of the virion and four form the nucleoprotein core (14). During the process of virion budding, the particles also incorporate many cellular components, including nucleic acids (8), lipids (35), histocompatibility antigens (6), adenosine triphosphatases (11), kinases (36, 43), and actin. Whereas the cellular lipids and nucleic acids are both specific and required for virion structure, the role of the other cell components in virions is unknown.

We have reported the isolation and characterization of a defective mammalian oncovirus (KW virus), which is comprised of only one virion-encoded structural protein (33). Instead, these particles contain several novel proteins incorporated from the rat host cell membranes. The cellular and viral expression of three of these proteins (gp110, gp55, p20) has been partially characterized (38). gp110 and p20 are transformation related. In contrast, gp55, a 55,000-dalton glycoprotein, appeared common to several rodent retrovirus preparations, but was unrelated to virus transformation. We now report the purification of gp55 and describe its biochemical features and quantitative distribution in viruses, in cells, and in tissues of rats and mice. The quantitative data confirmed and extended our previous findings and demonstrated 50- to 100-fold higher concentrations of gp55 in viruses than in cells.

MATERIALS AND METHODS

KW (NRK) cells and KW particles. The initial isolation and biological characterization of the KW-transformed rat cell line and the particles it produces have been described previously (33). The Kirsten sarcoma virus (KiSV)-transformed, woolly monkey leukemia virus-infected cells produce only replication-defective, pseudotype particles (KW virus) containing reverse transcriptase, woolly monkey leukemia virus p28, and transforming KiSV genes.

Other cells and viruses. All cells were grown at 37°C in Dulbecco-modified Eagle medium supplemented with 10% heat-inactivated fetal calf serum without antibiotics. Cell cultures did not contain mycoplasma as determined by aerobic and anaerobic culture techniques (Flow Laboratories Inc., Rockville, Md.). The AKT-8 cell line (41), an AKR xenotropic virus-transformed nonproducer mink fibroblast culture, MCF-247 virus-producing SC1 mouse cells, and AKR-6T xenotropic virus-producing CCL64 mink cells (15) were the generous gift of J. Hartley. The Hu8085 cell line, a human skin and muscle culture, was provided by A. Hackett. The HUK line, a clone of normal rat kidney (NRK) cells productively infected with F26 virus (4), which is an isolate of KiSV-Kirsten leukemia virus (KSLV) grown in NRK cells, was kindly provided by J. Bilello. The following cell lines and viruses were described previously (47): NIH Swiss mouse embryo culture (NIH/3T3); Osborn-Mendel NRK culture; BALB/c xenotropic virus 2; KiSV pseudotype of the NIH Swiss xenotropic class III virus and NZB xenotropic class III virus—all three propagated in human rhabdomyosarcoma cells, A667; Rauscher murine leukemia virus (RLV), propagated in JLS-V9 cells; the Rickard strain of feline leukemia virus; KSLV; and AKR murine virus. The 3T3 FL cells derived from an outbred Swiss mouse embryo culture and the D56 S^+L^- line, a transformed subclone infected with Moloney sarcoma virus, have also been described (49). 3T3 FL clones 23:28 and 23:32, isolated from a culture of 3T3 FL cells infected with B tropic virus (19), and spontaneously transformed nonproducer NRK cells were the generous gift of S. Kashmiri. RLV was also isolated from NRK cells, from human embryo kidney cells, and from NIH/3T3 cells as described earlier (45). The NS-1 suspension cultures of BALB/c plasmacytomas were kindly provided by C. Milstein (22), and the Kirsten-transformed nonproducer NIH/3T3 cells were a gift of S. Aaronson. Normal BALB/3T3 cells and KiSV-transformed nonproducer K234 cells are described by Strand and August (48). The 1255-B7 culture, a KiSV-transformed nonproducer NRK line from Klement (21); the Moloney-transformed S^+L^- (CCL64), a gift from P. Peebles (34); and lymphocytes from a patient with chronic lymphocytic leukemia from the Johns Hopkins Hospital were also used. V-NRK virus, a spontaneously released defective rat virus (27), was obtained from V-NRK cells, a gift of E. Scolnick. CCL64, a mink lung fibroblast line, was obtained from the American Type Culture Collection, and its virus-infected derivatives (NZB) CCL64, (BALB2) CCL64, (KSLV) CCL64, and (MCF-247) CCL64 were established in this laboratory. The J400 rat leukemia virus was propagated in NRK cells.

Purification of gp55 for radioimmunoassay. (i) Preparative gel electrophoretic method. The 55,000-dalton glycoprotein, gp55, was purified to electrophoretic homogeneity from KW virus as follows. Culture fluid harvested from confluent monolayers of KW cells was concentrated 20-fold with the Pellicon ultrafiltrator (Millipore Corp., Bedford, Mass.), and the concentrate was sedimented at 50,000 × *g* for 2 h. The pellet was suspended in a small volume of 20 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.6)–1 mM ethylenediaminetetraacetic acid (EDTA)–100 mM NaCl and isopycnically banded on a 15 to 60% (wt/vol) sucrose gradient at 200,000 × *g* for 190 min in a Sorvall vertical rotor. The virus band was removed from the gradient, diluted to a sucrose concentration of 15% (wt/vol), and pelleted for 130 min at 150,000 × *g*.

The virus proteins were suspended in 0.5 ml of 1 M Tris-hydrochloride (pH 8.2)–1 mM EDTA–100 mM NaCl–0.1% sodium dodecyl sulfate (SDS) and reduced by adding dithiothreitol to a final concentration of 50 mM. After incubation for 2 h at 37°C, the proteins were alkylated by the addition of iodoacetamide to a concentration of 0.1 M. This reaction mix was immediately and exhaustively dialyzed against 10 mM ammonium bicarbonate containing 0.1% SDS. The dialysate was then lyophilized.

The alkylated, lyophilized virus was solubilized in electrophoresis sample buffer (see below) and heated to 37°C for 1 h. Traces of ^{125}I-labeled molecular weight markers were added, and the entire sample was fractionated on a 4-ml, cylindrical, 3.5-mm-diameter, 5 to 20% polyacrylamide gradient gel, containing 0.1% SDS. After electrophoresis, the unstained, unfixed gel was sliced, and appropriate fractions were extracted overnight at 37°C into 10 mM ammonium bicarbonate with 0.1% SDS, 2 mM phenylmethylsulfonyl fluoride, and a trace of chloroform. The eluant was filtered through glass wool and stored on ice. The extract containing the gp55 was radiolabeled with iodine and tested for electrophoretic purity and antigenicity.

(ii) Chromatographic method. KW virus was purified as described above and was disrupted in lysis buffer containing 1% Triton X-100, 0.4 M KCl, 1 mM EDTA, 5 mM Tris-hydrochloride (pH 9.2), 2 mM phenylmethylsulfonyl fluoride, and 200 U of Trasylol per ml of extract. The suspension was incubated at 37°C for 10 min, then frozen in dry ice-methanol and thawed at 37°C three times. This solubilized virus was then exhaustively dialyzed against 20 mM Tris-hydrochloride (pH 7.6)–1 mM EDTA–100 mM NaCl with 0.2% Triton X-100 and clarified by centrifugation. Proteins which were precipitated by 30% ammonium sulfate were collected by centrifugation and suspended in deionized water and dialyzed against 20 mM Tris (pH 7.6)–100 mM NaCl–0.2% Triton X-100 containing 1 mM each of $CaCl_2$, $MnCl_2$, and $MgCl_2$. The dialyzed proteins were clarified by centrifugation and applied to a 1-ml concanavalin A–Sepharose column (Pharmacia Fine Chemical, Inc., Piscataway, N.J.), equilibrated in the same buffer. To prevent leaching of the lectin, the concanavalin A-Sepharose was cross-linked before use by treatment with 0.25% glutaraldehyde for 1 min at 25°C and 60 min at 0°C while stirring in 1 M phosphate buffer at pH 7.0. This procedure was modified (J. S. Krakow, personal communication) from a technique described for uncoupled lectins (29). The bound proteins were eluted with a buffer containing 50% ethylene glycol, 20 mM Tris-hydrochloride (pH 7.6), 1 M NaCl, 0.2% Triton X-100, and 0.2 M *α-methyl*-D-mannoside. The eluted proteins were dialyzed against 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.1)–20 mM NaCl–0.1% Triton X-100 and applied to a 1-ml heparin-agarose column equilibrated in the same buffer. The column was extensively washed, and the bound proteins were

eluted with 0.5 M NaCl in 20 mM Tris-hydrochloride (pH 7.6)–0.1% Triton X-100. During the purification, proteins in the column fractions were visualized with a microradioiodination and gel analysis procedure modified from Hunter (18) by Krakow et al. (manuscript in preparation).

Radioimmunoassay. Purified gp55 was labeled with ^{125}I as described by Hunter (18), except that the gp55 was separated from the reactants by dialysis. Radioimmunoassays and competition radioimmunoassays of competing proteins in cell, virus, and tissue extracts were conducted as described by Strand et al. (50).

Cell extractions. The proteins of tissue culture cells and homogenized tissues were solubilized in lysis buffer as described for disrupting KW virus (above). Viruses to be used in competition radioimmunoassays were disrupted by the addition of Triton X-100 to 0.4%. Proteins were determined by the methods of Lowry et al. (30) and Bradford (5) except during the chromatographic purification of gp55 when the technique of Schaffner and Weissmann (37) was used.

Immunoprecipitation gel electrophoresis, autoradiography, and fluorography. These procedures have been described elsewhere (38).

Antisera. Rabbit anti-KSLV serum and goat anti-woolly monkey leukemia virus serum were prepared as described (50). Goat antisera against a spontaneous rat lymphoma virus (anti-rat virus), the gibbon ape leukemia virus, the baboon leukemia virus, the RD-144 cat leukemia virus, the BALB murine xenotropic virus 2, rabbit immunoglobulin, pig anti-goat immunoglobulin serum, and hamster anti-simian virus 40 T antigen serum were provided by Roger Wilsnack under a contract from the Division of Cancer Cause and Prevention, National Cancer Institute. Antiserum against murine xenotropic leukemia virus envelope glycoprotein, gp70, was a gift of Stephen Kennel. Antisera against the purified RLV gp70, p30, p15, p12, and p10 and against the Gross murine leukemia virus p15E, p14, p12, and p10 were prepared in this laboratory (45, 47, 50).

RESULTS

Chromatographic purification of gp55. The gp55 was chromatographically purified from 10 liters of tissue culture fluid (Fig. 1 and Table 1). This procedure produced 30 μg of gp55 at greater than 90% purity with a final yield of 20% from the sucrose-banded virus. Much of the loss of gp55 occurred during ammonium sulfate precipitation, since some gp55 remained soluble. However, inclusion of this step was necessary to purify another KW virus-associated protein, p20 (39). An additional 6 μg of gp55 was recovered from the ammonium sulfate supernatant fraction at comparable purity. At pH 7.1, the binding of gp55 to the heparin-agarose affinity column was remarkably specific. At more basic pH's, gp55 was only partially bound, and at more acidic pH's, many other proteins also bound to this matrix. The avidity of binding of gp55 to concanavalin A-Sepharose was also noteworthy.

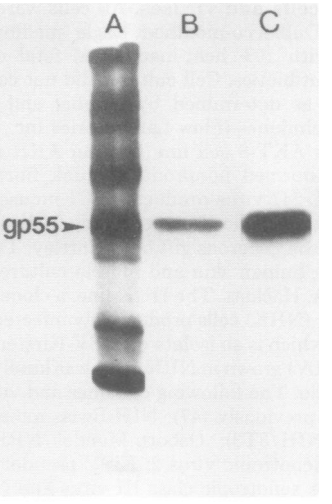

FIG. 1. *Purification of KW gp55 from KW virus. Samples were radioiodinated, trichloroacetic acid precipitated, fractionated by 12.5% SDS-polyacrylamide gel electrophoresis, and analyzed by autoradiography as described in the text. (A) Sucrose-banded, disrupted KW virus. (B) SDS-gel-purified gp55. (C) Chromatographically purified gp55. This photograph is a composite of three gel lanes aligned using several identical molecular weight markers for each lane.*

TABLE 1. *Purification of gp55*

Fraction	Total protein (μg)	gp55 (%)
Culture fluid (10 liters)	7×10^7	ND[a]
Pellet from concentrated culture fluid	5.5×10^4	0.5[b]
Sucrose-banded virus	3.5×10^3	4.4[b]
Ammonium sulfate pellet	8×10^2	ND
Concanavalin A-bound fraction	1×10^2	ND
Heparin-bound fraction	30	>90[c]

[a] Not done.

[b] Determined by competition radioimmunoassay using the purified gp55.

[c] Determined by measuring the radioactivity in the ^{125}I-labeled proteins cut from the gel.

α-*methyl*-D-Mannoside (200 mM), even in the presence of 1 M NaCl and 0.2% Triton, was only partially effective in eluting the gp55. Ethylene glycol was found to be necessary for complete elution.

gp55 could be further purified to 100% homogeneity by molecular sieve chromatography on Sephacryl S-200 in 0.5 M NaCl–0.1% Triton X-100–20 mM Tris-hydrochloride (pH 7.6); however, use of this column was not feasible for preparative work due to loss of protein. gp55 was eluted from this column with an apparent mo-

lecular weight of 150,000. This elution property can be attributed to Triton X-100 micells, which have an apparent molecular weight of approximately 90,000 (16). It is also possible that gp55 was in a dimeric form.

Preparative SDS-gel purification of gp55. Approximately 0.5 mg of KW virus was concentrated, reduced, alkylated, and fractionated by SDS-polyacrylamide gel electrophoresis. Since we had observed that gp55 migrated differently in nonreducing gels, reduction and alkylation of the KW virus proteins were necessary to insure that gp55 migrated as a 55,000-dalton protein. Approximately 25 μg of gp55 was obtained at greater than 95% purity, as assessed by gel electrophoresis (Fig. 1). The yield of protein from the gel, as measured by radioactive tracers, was greater than 98%.

Radioimmunoprecipitation assays of gp55. The origin and function of gp55 was analyzed by characterization of the antigenic reactivities of this protein. The purified gp55 was radiolabeled with ^{125}I to a specific activity of 10^4 cpm/ng and analyzed by immunoprecipitation assays with antisera prepared against retroviruses, purified retroviral proteins, and cellular proteins. The gp55 purified by both methods behaved nearly identically, with one exception as noted below. Therefore, all of the radioimmunoassay data presented here refer to results obtained with the gel-purified gp55.

Several different antisera prepared against rodent viruses grown in rodent cells precipitated 35 to 75% of the purified radioiodinated gp55. In contrast, antisera against nonrodent viruses, even at low dilution, were only able to precipitate 4 to 8% of the gp55. This low-titer reaction suggested the presence of a cross-reacting, nonrodent protein in these viruses. The presence of this analogous protein was confirmed by competition radioimmunoassay (below). Many other sera, including antisera against simian virus 40 T antigens, fetal calf serum proteins, mouse and rat histocompatibility antigens, Rauscher and xenotropic type C retrovirus gp70 envelope proteins, and retrovirus core proteins; normal serum from a variety of species; and monoclonal sera against three different 55,000-dalton membrane proteins (E. N. Hughes and J. T. August, J. Biol. Chem., in press; 42), did not precipitate the gp55. Therefore, gp55 appeared to be specifically associated with retroviruses, but was not related to any known retrovirus-encoded structural protein.

Competition radioimmunoassay for gp55. A competition radioimmunoassay using the purified radiolabeled gp55 enabled us to determine whether the presence of gp55 correlated with viral infection, transformation, or other cellular or viral functions. Goat anti-rat virus serum was used in the competition radioimmunoassays because of its high titer against gp55. At low dilutions it precipitated approximately 75% of the labeled protein, and at 10% of the input gp55.

A total of 25 cell lines representing normal, virus-infected, and transformed cells of four different species were screened for the presence of gp55 with the anti-rat virus serum (Table 2). Antigenic determinants cross-reacting with gp55 were present in low concentrations in every rodent cell line. Infection or transformation of a cell line did not affect the quantity of gp55 in that cell line. Nonrodent cells did not express significant amounts of cross-reacting antigens when assayed with this serum.

We also tested 14 purified virus particle preparations (Table 3). All viruses budding from rodent cells contained large amounts of gp55, at concentrations 50 to 100-fold higher than the host cells. The amount of gp55 in a virus preparation was dependent upon the cell line from which it budded. RLV propagated in NRK cells contained roughly three times more gp55 than RLV from NIH/3T3 or JLS-V9 (mouse) cells. RLV grown in human embryo kidney cells contained no detectable gp55. These quantities in the viruses correspond to the quantities of gp55 measured in rat, mouse, and human cells, respectively. Therefore, gp55 was associated with the host cells and not with a particular exogenous virus.

The cross-reacting protein in mouse cells was identified by immunoprecipitation also to be a 55,000-dalton protein. An anti-KSLV serum was made specific for the KW gp55 by solid-phase absorption with rat and mink cell extracts. This antiserum precipitated a 55,000-dalton protein from ^{125}I-labeled RLV grown in JLS-V9 cells.

Since retrovirus structural proteins are found in mouse serum (46), we also tested sera from a variety of mouse and rat strains for the presence of gp55. NZB mouse serum contained 7 ng of cross-reacting antigen in 10 μl. Sera of other strains (Sprague-Dawley, Wistar Furth, and Osborn-Mendel rats and BALB/c, 129 G IX^+, and 129 G IX^- mice) also contained competing antigen, although too little to be quantitated. NZB mouse serum was also found to be able to precipitate radiolabeled gp55 (Myerson and Scheinberg, unpublished data). Hence, NZB serum contained both gp55 antigen and antibody to the antigen.

The distribution of gp55 in the organs of both mice and rats was also quantitated by a competition radioimmunoassay (Table 4). The lymphoid tissues, spleen, and thymus contained the most gp55, followed by lung, ovary, and kidney. Other organs, including liver, brain, heart, and erythrocytes, contained either no gp55 at all or

TABLE 2. *Concentrations of gp55 in tissue culture cells*

Cell line	Cell characteristics	gp55 (ng per mg of protein[a])
Rat		
NRK	Normal	285
1255-B7	Virus-transformed nonproducer	333
KW	Virus-transformed defective	364
NRK-HTr	Spontaneously transformed	235
HUK	Virus-transformed producer	200
Mouse		
BALB 3T3	Normal	308
K-234	Virus-transformed nonproducer	133
NIH/3T3	Normal	100
(RLV) NIH	Virus-infected	142
Ki-NIH	Virus-transformed nonproducer	182
3T3 FL	Normal	111
3T3 FL clone 28	Virus-infected	67
3T3 FL clone 32	Virus-infected	57
D56	Virus-transformed nonproducer	133
NS1	Transformed spontaneous virus producer	200
Mink		
CCL64	Normal	<2
(BALB2) CCL64	Virus-infected	<2
(NZB) CCL64	Virus-infected	<2
(S^+L^-) CCL64	Virus-transformed nonproducer	<2
(KiSV) CCL64	Virus-transformed producer	<2
(AKR-6T) CCL64	Virus-infected	<2
AKT-8	Virus-transformed nonproducer	<2
(MCF) CCL64	Virus-infected	<2
Human		
CLL[b]	Leukemia cells	<2
Hu8085	Normal	<2

[a] Calculated from the point of 50% competition of unlabeled extract with ^{125}I-labeled gp55 in radioimmunoassays using gel-purified gp55 and anti-rat virus serum at 1:10,000 dilution. The sensitivity of the assay was approximately 2 ng.

[b] CLL, Chronic lymphocytic leukemia.

TABLE 3. *Concentrations of gp55 in type C viruses*

Virus	Host cell	gp55 (ng per mg of protein[a])
F26 (KSLV)	NRK	44,444
J400	NRK	36,364
KW	NRK	28,571
RLV	NRK	15,385
KSLV	NRK	14,285
HUK (KSLV)	NRK	12,500
RLV	NIH/3T3	6,667
RLV	JLS-V9	4,444
AKR	BALB 3T3	800
FeLV[b]	Cat	<1
RLV	Human	<1
BALB 2 xeno	Human	<1
KiSV (NIH xeno)	Human	<1
KiSV (NZB xeno)	Human	<1

[a] Calculated as in Table 2, footnote *a*.

[b] FeLV, Feline leukemia virus.

levels of protein below the sensitivity of this assay. Both species showed a similar pattern of expression.

The competition assays described above were predominantly rodent group specific. The slopes of the competition curves obtained with both rat and mouse cells were the same, indicating that the cross-reacting antigens were identical (data not shown). Therefore, the anti-rat virus serum detected shared rodent-specific antigenic determinants.

We also conducted competition radioimmunoassays with a much broader reacting antiserum, the anti-KSLV serum (Fig. 2). In this assay, the slopes of the competition curves decreased for rat, mouse, and nonrodent cell extracts, respectively. Compared with the competion curves obtained with rat cell extracts, approximately fourfold more protein was required for 50% com-

TABLE 4. *Concentrations of gp55 in mouse and rat tissues*

Tissue	gp55 (ng/mg of tissue protein)	
	Sprague-Dawley rat	BALB/c mouse
Spleen	80	69
Ovary	31	Not done
Thymus	25	61
Lung	15	47
Kidney	6	13
Liver	<1	<1
Brain	<1	<1
Heart	<1	<1
Erythrocytes	<1	<1

[a] Calculated as in Table 2, footnote *a*.

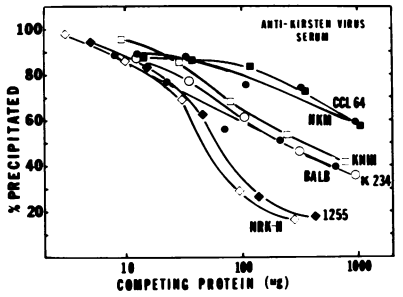

FIG. 2. *The rat type, rodent group, and interspecies antigenic determinants of gp55 as distinguished by anti-KSLV serum in competition radioimmunoassay. The assays were conducted as described in the text with anti-KSLV serum at a final dilution of 1:80 and 20 ng of purified ^{125}I-labeled gp55 in competition with unlabeled cell extracts added as indicated. CCL64: ■, normal mink; HKM: ●, KSLV-infected CCL64; BALB: ●, normal mouse; K-234: ○, KSLV-transformed nonproducer BALB; KNIH: □, KSLV-transformed nonproducer NIH/3T3 mouse cells; NRK-H: ◇, NRK; 1255: ◆, KSLV-transformed nonproducer NRK cell.*

petition when murine cell extracts were used. The nonrodent, mink cells competed nearly 50%. Human cells (not shown) behaved similarly. Therefore the anti-KSLV serum was able to detect rat type-specific and interspecies antigenic determinants on gp55 in addition to the rodent group-specific antigens. The slopes of the curves differed because there were gp55 rat type-specific determinants not present in the analogous protein of murine, mink, and human cells and gp55 rodent group-specific determinants not present in the mink and human cells. These data are in contrast to the competition curves obtained when the anti-rat virus serum was used, where the antigens other than rodent group specific were not detected, and identical affinity was observed with both murine and rat competing proteins.

As we had observed when using the anti-rat virus serum, the assays using anti-KSLV serum showed no apparent effect of transformation on the amount of gp55 expressed in cells. This was observed in cells of all four species.

Since neither rat type- nor rodent-group-specific antigens were found in mink cells infected by rat- and mouse-derived viruses (Fig. 2 and Table 2), gp55 did not appear to be encoded by genes in these viruses.

Assays conducted with the chromatographically purified gp55 and anti-KSLV serum also detected the rat type- and rodent group-specific antigens, but not the interspecies antigenic determinants. It is possible that the interspecies sites were exposed to the antibodies only on the gel-purified protein because of the denaturing effect of SDS.

DISCUSSION

gp55 was purified from KW virus by two different techniques to yield homogeneous protein preparations of similar antigenicity. Use of a preparative gel electrophoretic technique enabled us to rapidly obtain protein suitable for use as a probe in competition radioimmunoassays. During the chromatographic purification, some interesting properties of the polypeptide were revealed. We had previously observed that gp55 was a glycoprotein (38) and, consequently, affinity chromatography on concanavalin A-Sepharose was a very effective purification step. Two-dimensional gel electrophoretic analysis of the protein (unpublished data) showed it to be a slightly acidic, microheterogeneous protein with a pH ranging from 6.0 to 7.4. Microheterogeneous patterns are commonly observed with glycoproteins fractionated by this technique. gp55 bound specifically to heparin-agarose, which allowed us to purify it from the other glycoproteins. Heparin has been shown to bind deoxyribonucleic acid-binding proteins (9) and extracellular matrix proteins (53). The significance of gp55's binding to heparin is not known. gp55 also had hydrophobic regions since it bound to both phenyl-Sepharose (Pharmacia) and silica glass beads (Cabot) (data not shown). We had previously reported that gp55 was associated with purified cell membranes isolated by polyethylene glycol 6000-dextran T 500 phase fractionation (38). The hydrophobicity of gp55 is consistent with its association with cell membranes. However, gp55 does not appear to be easily accessible at the external cell surface as it is not labeled by lactoperoxidase (38) nor tritiated borohydride (unpublished data). Furthermore, in contrast to the rat cell extracts, live rat cells do not absorb the anti-gp55 reactivity from the anti-rat virus serum (unpublished data).

Study of the purified, radiolabeled gp55 by radioimmunoprecipitation assay confirmed and

extended our earlier immunoprecipitation analysis of proteins associated with virions (38). Only antisera prepared against rodent type C viruses grown in rodent cells were strongly reactive with the purified gp55. Quantitative study by competition radioimmunoassay indicated that gp55 was not primarily associated with the type C virus itself but with the cell from which the virus buds. This does not rule out an endogenous virus origin for the protein, however.

gp55 was expressed constitutively in normal tissue culture cells of four different species tested. Transformation or infection of these cells by a number of different viruses did not dramatically change its quantity. The low quantities of gp55 in tissue extracts (<0.0001 to 0.008%) suggested that this was not a common cytostructural protein. Antisera to three 55,000-dalton cell surface proteins and to rodent histocompatibility antigens did not react with gp55. Moreover, purified microtubules (the generous gift of Douglas Murphy) did not cross-react with gp55 in radioimmunoassays (data not shown).

Our earlier studies had already indicated that the qualitative parameters of the expression and cellular location of gp55 in rat cells were not altered upon transformation (38). Therefore, it is unlikely that gp55 is the transformation-related 53,000-dalton protein described by DeLeo et al. (10). Furthermore, gp55 did not react with antiserum to simian virus 40 T antigen, which precipitates a 55,000-dalton T-antigen-associated protein (7, 23, 24, 28, 31).

The large amount of gp55 in virion preparations, sometimes greater than 4% of the virus protein, was striking. Studies of Aoki et al. (1), Aoki and Takahashi (2), Dorfman et al. (12), and Witte and Weissmann (52) indicate that the site of virion budding is unique and that incorporation of cell membrane proteins is extremely selective and specific for virus-encoded proteins. Therefore, the 50- to 100-fold concentration of gp55 by retroviruses suggests a nonrandom association of this particular membrane protein with the virion. In contrast, p20, another membrane-associated protein detected in the KW virus, was found to be only 10-fold concentrated in virions as compared to cells, and this increase is more consistent with a nonspecific incorporation of membrane protein into virions (39).

These data are consistent with two possible functions for gp55. It may be involved in the packaging or budding of particles or may be an endogenous virus glycoprotein. A defective endogenous type C virus RNA has been detected in a variety of rat and mouse fibroblast cells in culture which could potentially encode this protein (3, 13, 17, 20, 40). Furthermore, there were several resemblances between gp55 and the other retrovirus envelope glycoproteins: (i) the lymphoid and epithelial tissue distribution of gp55 is similar to that of gp70 (26); (ii) gp55 was found in the sera of rodents, especially in the NZB mouse, as is gp70 (46); (iii) antibodies to gp55 were found in NZB mouse serum, as are antibodies to gp70 (32); (iv) several of the known retrovirus envelope proteins are glycoproteins of 55,000 daltons (51); (v) the antigenic spectrum of gp55 demonstrating a wide interspecies variation of the polypeptide is consistent with the antigenic characteristics that have been described for known virus envelope glycoproteins (44) and not consistent with the more conserved determinants of virus core proteins or cellular cytostructural proteins; and (vi) the quantity of gp55 in some virions is equivalent to the quantity of gp70 commonly found in virions.

We conclude that gp55 is a constitutively expressed cell membrane glycoprotein which is unusual both in its tissue distribution and in its association in high concentrations with type C retroviruses.

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