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Role of Carbohydrate in Determining the Immunochemical Properties of the Major Glycoprotein (gp71) of Friend Murine Leukemia Virus

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Treatment of Friend leukemia virus gp71 with protease-free glycosidase enzymes results in removal of the major portion of the carbohydrate without affecting the amount of protein present. The digested material migrates as a protein of about 60,000 to 65,000 molecular weight on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Analyses of the serological properties of gp71 after enzyme treatment indicated that the type, group, and interspecies determinants were not destroyed. In contrast, treatment with proteolytic enzymes led to the complete destruction of the gp71 molecule, including the total elimination of its serological reactivity as measured by direct and competition radioimmunoassay and by a serum cytotoxicity assay. We conclude that the carbohydrate portion of gp71 is not of major significance in defining the antigenic determinants of this viral glycoprotein.

The major structural component of the oncornavirus surface is a glycoprotein of high molecular weight. In avian viruses this molecule (gp85) determines a number of key properties including virus strain specificity, host range, interference, and neutralization pattern (4). In murine viruses, antisera to the purified glycoprotein (gp71) neutralize virus infectivity, but with somewhat less strain specificity than is observed with avian agents (16, 30). The purified murine virus glycoprotein can also interfere with virus infection and has the added capability of hemagglutinating erythrocytes (16, 34, 35). In addition to these properties, both the avian (L. Rohrschneider, H. Bauer, and D. P. Bolognesi, Virology, in press) and murine (G. Hunsmann, M. Claviez, V. Moennig, H. Schwarz, and W. Schäfer, Virology, in press; H. Schwarz, G. Hunsmann, V. Moennig, and W. Schäfer, Virology, in press; 16, 30, 35) molecules possess multiple antigenic determinants when examined in standard serological tests including immunodiffusion, complement fixation, radioimmunoassay, cytotoxicity, and immunoelectromicroscopy. These have been denoted as the group (shared by viruses of the same species), type (unique to an individual virus strain), and interspecies (shared by viruses of different mammalian species) determinants (2). Recent work has shown that gp71 can be easily released from the surface of virus-producing cells (D. P. Bolognesi, A. J. Langlois, and W. Schäfer, Virology, in press). In addition, a molecule similar to gp71 is found in high concentration in various organs in the mouse (R. Lerner, personal communication). As a soluble antigen this component is probably responsible for the induction of natural antibody in mice to type C oncornaviruses and may also be involved in other interactions with the host immune system.

Other classes of enveloped viruses likewise possess glycoproteins on their surface with generally similar properties and functions. Until recently, little was known in terms of the relative contribution of the protein and carbohydrate to the various activities of virus glycoproteins. Studies with vesicular stomatitis virus suggest that although carbohydrate does not contribute to the serological specificity of the molecule, neuraminic acid plays a key role in the infection process (26). Studies with neuraminidase-containing viruses (e.g., myxoviruses and parainfluenzaviruses) have demonstrated the presence of viral envelope sugars which specifically bind to neuraminic acid receptors on the host cell surface (11). However, the role of the nonterminal sugar groups, which constitute the majority of the carbohydrate portion of these virion glycoproteins, has not yet been determined. The present study was undertaken

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to determine how the carbohydrate portion influences the various physical and immunological properties of the gp71 major glycoprotein of the murine type C oncornaviruses. This work serves as a prelude to detailed investigations aimed at determining the principal reactive groups of this important molecule and how these relate to its complex biological functions.

MATERIALS AND METHODS

Viruses. Friend leukemia virus (FLV) was obtained from the Eveline cell line, which is derived from the STU mouse strain and is capable of growing either in suspension or in monolayer culture (E. Seifert, M. Claviez, H. Frank, G. Hunsmann, H. Schwarz, and W. Schäfer, Z. Naturforsch., in press). Avian myeloblastosis virus (AMV) was isolated from the plasma of leukemic chickens. Viruses were purified by velocity and equilibrium density gradient centrifugation as previously described (6).

Radioactive virus. Eveline cell cultures were grown in Eagle minimal essential medium (MEM) with 25% of the normal glucose concentration and containing 20 μCi of [^{3}H]glucosamine (2.41 Ci/mmol, New England Nuclear, Boston, Mass.) per ml. Virus was harvested after 24 h of labeling and purified to homogeneity. Virus was also labeled in the presence of both [^{3}H]glucosamine (as above) and 2 μCi of a ^{14}C-labeled amino acid mixture (0.1 mCi/mmol, New England Nuclear) per ml in medium reduced in glucose (25%) and amino acids (5%).

Preparation of purified viral components. FLV gp71 was prepared by the method described by Moennig et al. (21) employing osmotic shock for release of the antigen followed by density gradient centrifugation, affinity chromatography on concanavalin A-agarose columns, and Sephadex filtration. AMV gp85 was obtained by gel filtration in guanidine-hydrochloride followed by ion exchange chromatography as described recently (Rohrschneider et al., in press). FLV p30 was purified by gel filtration in guanidine-hydrochloride (13).

Antisera. Antisera to FLV gp71 were prepared in rabbits or goats as described recently (Hunsmann et al., in press). Rabbit antisera to FLV p30 or goat antisera to disrupted feline leukemia virus (FeLV) were also used (13). Absorptions of sera with soluble antigens were carried out by mixing one part antigen (0.5 mg/ml) with nine parts of appropriately diluted serum (in Eagle MEM without serum) and incubating overnight at 4 C. The mixture was then centrifuged at 8,000 $\times$ g for 3 h, and the absorption was repeated with one part of fresh antigen. Control unabsorbed sera were treated in parallel with MEM (without serum) in place of antigen. Absorptions of sera with intact cells utilized cells grown in suspension which were prewashed one time with phosphate-buffered saline. Absorption was carried out with 10^7 cells/0.1 ml of serum for 1 h at 37 C and overnight at 4 C. The cells were removed at 3,000 rpm for 30 min, and the absorption was repeated once more.

Preparation of glycosidase enzymes. An enzyme preparation containing β-galactosidase, β-N-acetylglucosaminidase, and endo-β-acetylglucosaminidase D activities was prepared essentially as previously described (1, 22). Crude enzyme, obtained by ammonium sulfate fractionation of the culture medium in which *Diplococcus pneumoniae* was grown, was further purified by Sephadex G-200 column chromatography. β-Galactosidase and β-N-acetylglucosaminidase activities were assayed by the use of the appropriate p-nitrophenylglycosides, with a unit of enzyme activity defined as the release of 1 μmol of p-nitrophenol per minute. Concentrated material from the G-200 column contained 0.067 U of protein β-N-acetylglucosaminidase per mg (0.72 U/ml) and 0.006 U of protein β-galactosidase per mg (0.062 U/ml). Endo-β-N-acetylglucosaminidase D activity was ascertained by the use of [^{14}C]acetyl-Asn-(GlcNAc)$_2$-(Man)$_5$ (kindly donated by Takashi Muramatsu, Department of Biochemistry, Kobe University Medical School, Kobe, Japan) as a substrate. Endoglycolytic cleavage was determined by the appearance of a [^{14}C]acetyl-Asn-glycopeptide with an R_f in high-voltage paper electrophoresis (17) and molecular weight consistent with [^{14}C]acetyl-Asn-GlcNAc. The mixture of exoglycosidases was used because of the resistance of some glycopeptides to the endoglycosidase activity in their absence.

Treatment of viral components with enzymes. FLV gp71 (50 μg) was incubated with 10 μl of the glycosidase enzyme mixture for 16 h at 37 C in phosphate-buffered saline, pH 7.2 (final volume 100 μl). Iodinated FLV gp71 and p30 and AMV gp85 (each approximately 10 ng) or [^{3}H]glucosamine-labeled FLV (about 10 μg) were treated with a similar amount of enzyme in the same fashion. For neuraminidase treatment, FLV gp71, p30, or [^{3}H]glucosamine-labeled FLV were incubated with 10 U of highly purified enzyme (*Vibrio comma* [*cholerae*]; kindly provided by W. Schwick; Behringwerke, Marburg, Germany; 500 U/ml) for 2 h at 37 C (final volume, 50 μl) at pH 7.2.

Treatment of purified viral components or of labeled virus with trypsin (Sigma) or Pronase (Calbiochem, San Diego, Calif.) (each at 0.1 mg/ml final concentration) was for 2 h at 37 C at pH 7.2. After treatment with both glycosidases and proteases, enzyme activity was blocked by addition of an excess of fetal calf serum, which serves as a substrate for all the enzymes.

Gel electrophoresis. Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) was carried out as previously described (6). Protein was detected by staining with Coomassie blue (CB) and carbohydrate with periodic acid-Schiff. The procedure used for analysis of radioactive gels has also been dscribed (6). In each case the radioactive samples were co-electrophoresed with unlabeled FLV, and the gel was split and stained with CB and periodic acid-Schiff to determine the position of the viral proteins and glycoproteins. The relative migration of the labeled peaks was determined in reference to known viral component markers.

Radioimmunoassay. The double antibody radioimmunoassay procedure used was that described by Strand and August (29). Polypeptides or glycoproteins were labeled with ^{125}I by the method described

by Greenwood et al. (14). The specific activity of the labeled components ranged from 5×10^3 to 2×10^4 counts/min per ng of material.

Between 1 and 5 ng of ^{125}I-labeled p30 or gp71 in a 10-μl volume was used in the assay. In direct radioimmunoassays, normal serum (30 μl) and immune serum (10 μl) were added and incubated at 37 C for 3 h and at 4 C overnight. All dilutions were made in radioimmunoassay buffer (20 mM Tris-hydrochloride, 100 mM NaCl, pH 7.6, and 1 mM EDTA containing 2 mg of bovine serum albumin per ml). Antiserum to the appropriate immunoglobulins (30 μl) (Cappel Laboratories, Downingtown, Pa.) was then added and incubated for 1 h at 37 C and 3 h at 4 C. Immunoprecipitates were collected by centrifugation (8,000 $\times g$, 2 min), washed twice with radioimmunoassay buffer (without bovine serum albumin) and counted in a Packard Autogamma Counter (Packard Instrument Co., Inc., Downers Grove, Ill.).

In the competition assay, increasing amounts of antigen (unlabeled) were incubated with a limiting antibody dilution (10 μl) and normal serum (30 μl) for 2 h at 37 C. The limiting antibody dilution, determined by direct radioimmunoassay, precipitated 50% of the respective labeled antigen. A standard amount of labeled antigen (1 to 5 ng in 10 μl) was then added and incubated for an additional 1 h at 37 C. After overnight incubation at 4 C, the samples were processed as described above.

Cytotoxicity assay. The [^{14}C]nicotinamide (NA) release assay, originally described by Martz et al. (20) as an assay for cell-mediated cytotoxicity, and modified by R. Kurth and G. Medley (Immunology, in press) for use as a microcytotoxicity test for humoral antibodies, was employed in this study with some further modifications. Briefly, between 2×10^3 and 8×10^3 target cells were seeded in each well of a Terasaki plate (Falcon microtest plate no. 3034, Oxnard, Calif.) in 10 μl of MEM supplemented with 10% fetal calf serum and 25 μCi of [^{14}C]NA (60 mCi/mmol, Amersham, England). For Eveline target cells optimal seeding was obtained with 4.3×10^3 cells/well. One-half milliliter of medium without serum was placed around the edge of the plate to reduce drying out of the wells. The cells were then incubated at 37 C in a humidified atmosphere of 95% air-5% CO_2 for approximately 24 h, at which time they had just reached confluence. Before use the cells were washed three times with ice-cold medium without serum by flooding the plate (~10 ml) and rocking on a slow shaker for 5 min. Wash fluid was aspirated from the plate and wells, and 5 μl of the appropriate serum dilution was added to triplicate wells. The plates were then incubated for 15 min at 37 C in a CO_2 incubator, and 10 μl of rabbit C′ (Pel Freeze, Rogers, Ark.) diluted 1/6 was added to appropriate wells. The plates were incubated again for 15 min at 37 C in a CO_2 incubator, and 5 μl of fluid was removed from each well for scintillation counting in Aquasol (New England Nuclear). Samples were counted in a Beckman LS-350 β-irradiation counter (Beckman Instruments, Palo Alto, Calif.).

Maximum release of radioactivity was determined by the addition of 15 μl of 0.5% Triton X-100 per well at the start of the first incubation. All experiments included C′ and medium controls (medium without serum used for all dilutions and blanks), and results are expressed as percentage specific release, where: percentage of specific release = [counts per minute test − counts per minute background]/[counts per minute maximum lysis (Triton X-100) − counts per minute background]. Background is either C′ control or medium control, and percentage specific release $\geq$ 20 is taken to reflect significant killing.

RESULTS

Treatment of glycoproteins with glycosidase enzymes. FLV gp71 isolated as described previously (21) was treated with the glycosidase enzyme mixture (see above) and analyzed by SDS-PAGE (Fig. 1). The untreated molecule migrated to the expected position on the gel (21) and could be stained with both CB and periodic acid-Schiff. After treatment with the enzymes, the material stained with CB is essentially preserved but migrates more rapidly, and the band is slightly broader than the untreated sample. In contrast, most of the periodic acid-Schiff-stainable material is removed after enzyme treatment, but that which remains is coincident with the more-rapidly migrating protein band. At the concentration employed the glycosidase enzyme proteins are detectable on the gel as a heterogeneous band spanning most of the gel below the position of untreated gp71 and the digested molecule (Fig. 1). No periodic acid-Schiff-stainable material was evident in the enzyme preparation in the region of gp71 or digested gp71, although some was present in apparently aggregated form at the very top of the gel.

Similar results were obtained when AMV gp85 was treated with the enzymes (Fig. 2). Essentially no loss of iodinated material (primarily protein) was evident after digestion, although the band migrated more rapidly and heterogeneously than the untreated glycoprotein.

These results indicate that the activity of the enzymes was directed primarily toward the carbohydrate portion of the glycoproteins and did not affect the integrity of the protein. This is consistent with other studies with this enzyme preparation which provided evidence for the absence of protease activity (22). Further examination of this question was undertaken by treatment of a viral protein, p30, with the glycosidases under the same conditions as the glycoproteins. As shown in Fig. 3, there is no effect on the amount or migration properties of p30 relative to the untreated sample.

Treatment of intact virus with various en-

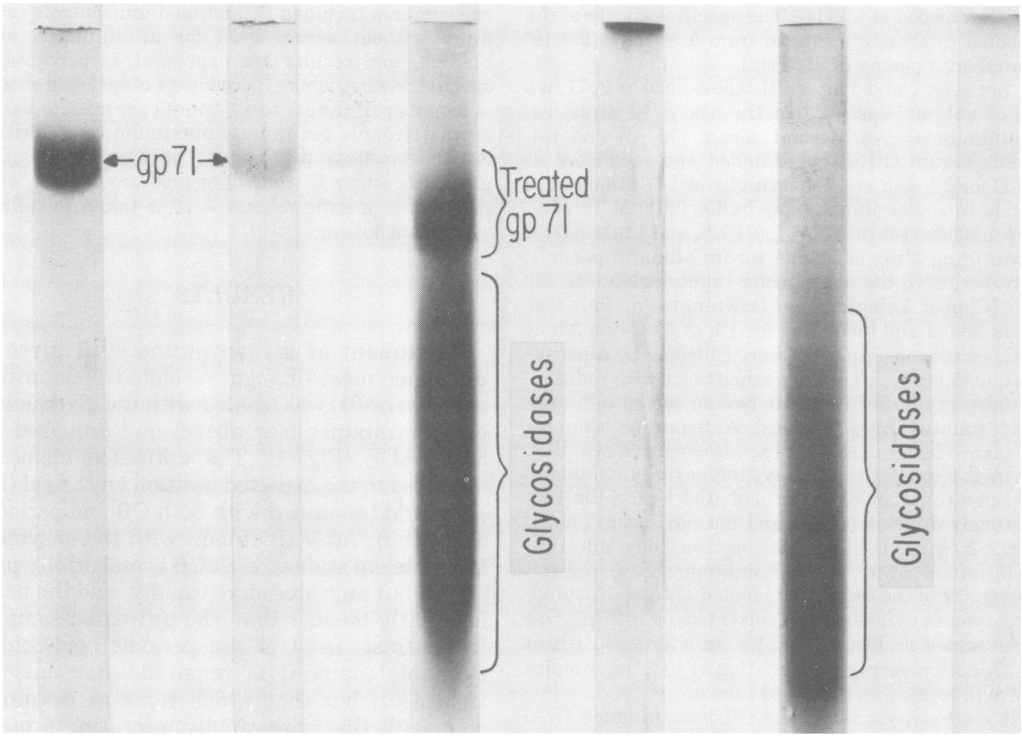

FIG. 1. *Treatment of gp71 with glycosidase enzymes. Purified gp71 (50 μg) was incubated with the glycosidases as described and electrophoresed on SDS-PAGE gels. The gels were sliced longitudinally and stained with CB (protein) and periodic acid-Schiff (carbohydrate). From left to right: gp71 (CB), gp71 (periodic acid-Schiff), gp71 treated with 50 μl of glycosidase preparation (CB), gp71 treated with 50 μl of preparation (periodic acid-Schiff), 20 μl of glycosidase preparation alone (CB), 20 μl of glycosidase preparation alone (periodic acid-Schiff). The control glycosidase enzymes were electrophoresed separately and approximately 10% longer than the other preparations shown.*

zymes. To assess the effect of the glycosidase mixture and other enzymes on the viral glycoproteins, [^{3}H]glucosamine-labeled intact FLV was employed (Fig. 4). Consistent with other studies (Bolognesi et al., in press; 21), the majority of the glucosamine label is incorporated in the virus as material migrating like gp71 with a minor amount appearing in a faster migrating component (gp45), as well as some at the bottom of the gel which probably represents glycolipid (25). That the major peak in fact represents gp71 could be shown by complete immunoprecipitation of this material with anti-gp71 antiserum (Bolognesi et al., in press).

Treatment of intact virus with the glycosidases removed about 80% of the glucosamine-labeled material from gp71 (Fig. 4). The gp71 component appears to be replaced by a much reduced and slightly broader peak which migrated to a position intermediate between gp45 and gp71. Most of the original gp45 material likewise appears to be lost, although some may be masked by the new peak. Together with the results in Fig. 1, it seems reasonable to assume that the new peak represents all or most of the protein in association with a small proportion of the original carbohydrate. This component appears to migrate as a protein of about 60,000 to 65,000 molecular weight; however, because of the uncertainty in the molecular weight of gp71 as determined by SDS-PAGE (9), it is not possible to directly correlate the increase in migration with a loss in true molecular weight.

Treatment of the virus with neuraminidase did not appreciably alter the migration behavior of gp71 or gp45. The slight decrease in the amount of label may be significant since a similar effect was observed by periodic acid-Schiff staining (not shown). On the other hand, treatment with proteolytic enzymes removes most of the glucosamine label, leaving a variety of fragments scattered throughout the gel which probably represent glycopeptides (Fig. 4).

Analysis of enzyme-treated FLV components by radioimmunoassay. The effect on the serological properties of gp71 and p30 after

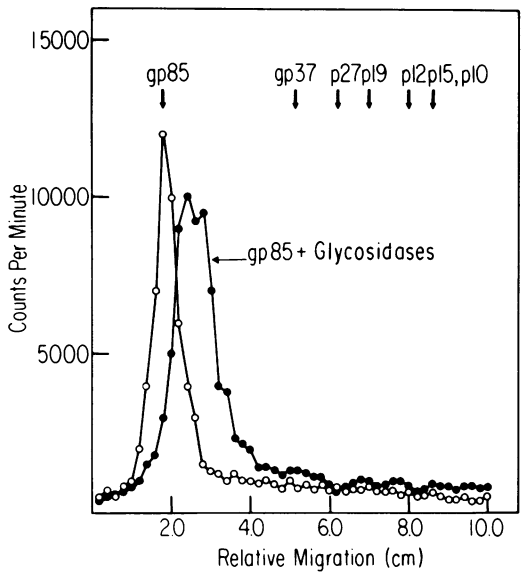

FIG. 2. *Treatment of AMV gp85 with glycosidase enzymes. Approximately 10 ng of [^{125}I]AMV gp85 was treated with glycosidases (●), whereas a corresponding amount was similarly incubated but not treated (○). Thereafter each sample was mixed with 100 μg of AMV and electrophoresed on SDS-PAGE. The arrows indicate the major AMV structural proteins which serve as reference markers. The position of AMV gp85 was established by periodic acid-Schiff staining, and that of gp37 and the remaining proteins was established by CB staining (7).*

treatment with various enzymes was determined by radioimmunoassay analyses. After digestion of the test antigens, the enzyme activity was blocked by addition of excess fetal calf serum. Under these conditions the serological tests employed were not affected by the presence of the enzymes used (see Fig. 6 and 7).

When antiserum to gp71 was used, essentially complete precipitation of iodinated gp71 was obtained after digestion by the glycosidase mixture or neuraminidase (not shown), and the precipitation curve is indistinguishable from that of the untreated sample (Fig. 5A). In contrast, the capacity to precipitate gp71 was lost when the molecule was treated with Pronase. The result obtained with antiserum prepared against disrupted FeLV, which reacts with the interspecies determinant of FLV gp71, were generally similar except that slightly less material was precipitated from the glycosidase-treated gp71 at high serum concentrations. This could represent a minor effect on the interspecies determinant of gp71. Analysis by SDS-PAGE of material immunoprecipitated with anti-FLV gp71 antiserum after glycosidase treatment revealed a single species migrating like the digested material in Fig. 1 and 4 (not shown).

Treatment of p30 with the glycosidases likewise did not affect the serological properties of the molecule (Fig. 5B). In this case no reduction whatsoever was seen in the interspecies assay, indicating that the effect seen with gp71 is not an artifact related to the use of the anti-FeLV antiserum. As expected, treatment of p30 with Pronase eliminated all antigenic activity.

Additional analyses of endoglycosidase-treated gp71 were carried out by competition radioimmunoassay. As expected from the above results, the treated molecule was as effective as the native gp71 in competition for gp71 antibodies (Fig. 6A). In the interspecies assay (anti-FeLV serum) the treated sample was slightly less effective in competition, although complete competition was achieved at high protein levels (Fig. 6B). This is in accord with the earlier suggestion of a possible slight effect by the enzyme on the interspecies region of gp71.

To determine the effect of enzyme treatment on the type-specific determinant of gp71, rabbit antiserum to gp71 was absorbed exhaustively (see above) with STU cells actively producing Gross leukemia virus (sharing group and interspecies determinants with FLV). The absorbed serum reacted with gp71 in radioimmunoassay, but the titer was reduced 50-fold. In a competition assay FLV competes effectively for the antibody, whereas the Gross virus does not, indicating that the serum is type specific for FLV (Fig. 7). When preparations of gp71 treated with var-

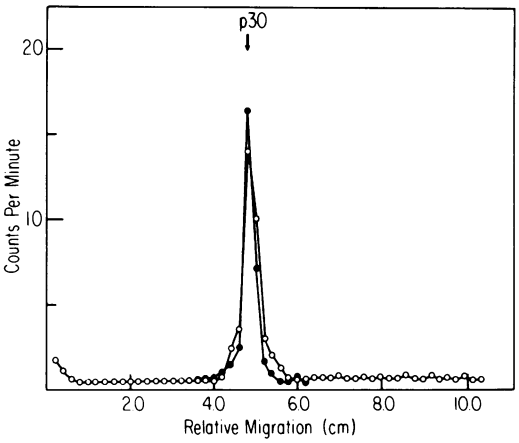

FIG. 3. *Treatment of p30 with glycosidase enzymes. FLV p30 labeled with ^{125}I was incubated with the glycosidase enzyme mixture (●) and co-electrophoresed on SDS-PAGE gels with purified unlabeled p30 (○). Prior to analysis of radioactivity the gels were stained with CB to determine the position of p30 on the gel (arrow).*

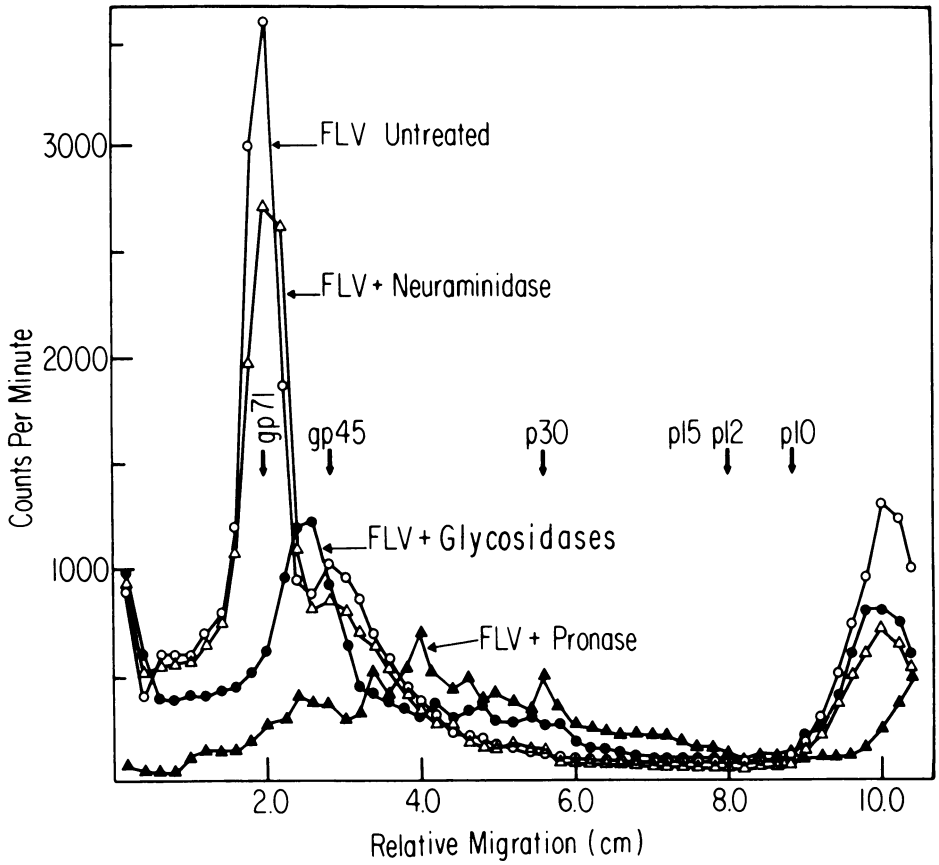

FIG. 4. *Treatment of labeled FLV with various enzymes. The virus was grown in the presence of [^{3}H]glucosamine and purified on density gradients. Aliquots of purified virus were incubated with the glycosidase mixture (●), neuraminidase (△), and Pronase (▲) as described and electrophoresed on SDS-PAGE in comparison to untreated virus (○). Each sample was co-electrophoresed with unlabeled FLV, and the gels were stained with CB and periodic acid-Schiff prior to analysis of radioactivity. The position of the labeled bands is plotted in terms of migration relative to the stained viral proteins indicated by arrows.*

ious enzymes were tested for competition with this serum (Fig. 7), there was no noticeable effect with either the glycosidase mixture or neuraminidase.

Taken together, these results indicate that the common antigenic determinants of gp71 (type, group, and interspecies) measured by radioimmunoassays are not significantly affected by removal of most of the carbohydrate but are essentially destroyed after proteolytic digestion.

Analysis of enzyme-treated gp71 by cytotoxicity assays. The possible role of the sugar residues of the gp71 molecule in determining the antigenic sites involved in C′-dependent killing of virus-producing cells was also examined. Titration of the goat antiserum to FLV gp71 in the [^{14}C]NA release assay indicated that this serum is extremely potent in lysing Friend virus-producing Eveline cells, demonstrating a 50% end point at greater than 1/4,000 (Fig. 8). On the basis of these results it was decided to use a dilution of 1/500 for the subsequent gp71 absorption studies.

The goat antiserum was diluted 1/500 in MEM without serum and absorbed as described above with the various preparations of enzymatically treated FLV gp71. Controls included unabsorbed goat antiserum (incubated with medium alone) and antiserum absorbed with gp71 which had not been treated with enzymes. Figure 9 demonstrates that absorption with the nontreated gp71 completely eliminated the cytotoxic activity of the goat antiserum on Eveline cells. Whereas treatment with either of the proteases completely removed the ability of gp71 to block the cytotoxicity reaction, treatment with the glycosidase mixture or neuramin-

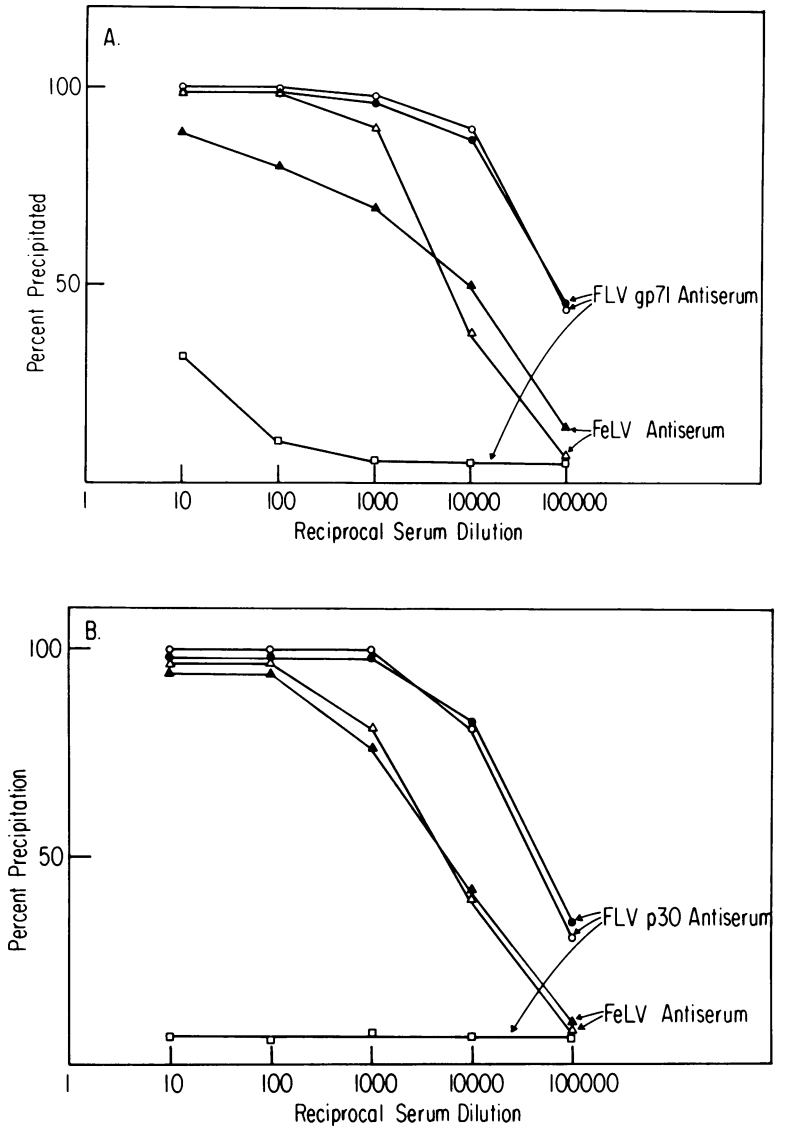

FIG. 5. *Precipitation of p30 and gp71 in radioimmunoassay after enzyme treatment. Iodinated p30 (A) and gp71 (B) treated with glycosidase enzymes (● or ▲), Pronase (□), or untreated (○ or △) were analyzed by direct radioimmunoassay employing antisera directed against the homologous FLV polypeptide (type, group, interspecies) or prepared against disrupted FeLV (interspecies).*

idase had little or no effect on the absorptive capacity of the gp71 molecule. Also included in Fig. 9 are controls which demonstrate that the enzyme preparations themselves (in the presence of C' and fetal calf serum) have no significant cytotoxic activity on the Eveline cells. These results indicate that the antigenic determinants being detected by the goat anti-gp71 antiserum which are involved in the cytotoxicity reaction do not require the presence of extensive sugar moieties and thus parallel closely the results obtained in the radoimmunoassay. Subsequent experiments, which will be described in detail elsewhere (J. J. Collins and D. P. Bolognesi, manuscript in preparation), demonstrate that the interspecies determinants involved in the killing of Eveline cells by goat anti-FeLV antiserum are also not affected by treatment with the glycosidase mixture or neuraminidase, whereas they are completely destroyed by treatment with trypsin or Pronase.

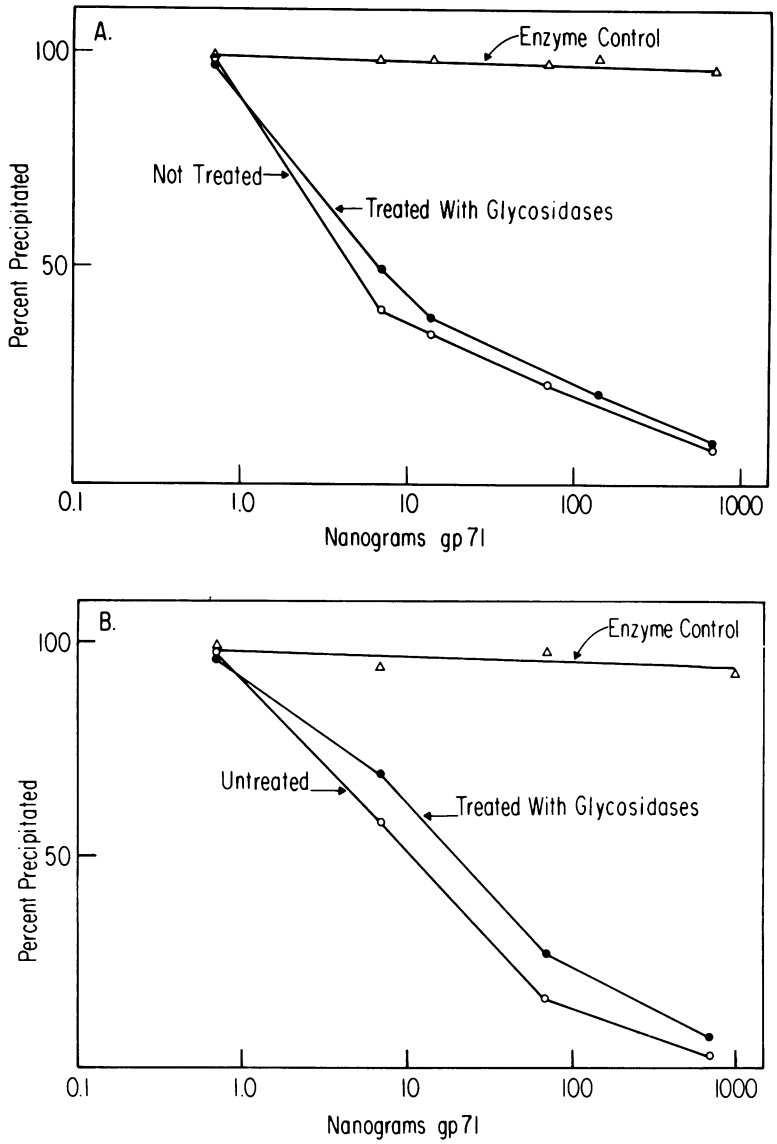

FIG. 6. *Competitive radioimmunoassays with enzyme-treated gp71. Unlabeled gp71 (1 mg/ml) treated with the glycosidase mixture* (●) *or untreated* (○) *was tested for its ability to compete with untreated* ^{125}*I-labeled gp71 for anti-FLV gp71 antiserum* (*A*) *or anti-FeLV antiserum* (*B*). *Quantities of enzyme corresponding to those present in the gp71 samples were also tested for their effect on precipitation of the antigen* (△).

DISCUSSION

Treatment of gp71 with the glycosidase enzyme mixture results in removal of most of the glucosamine label and the periodic acid-Schiff-stainable material. Although we have not determined the precise amount of carbohydrate which has been removed, the above two measurements clearly indicate that it is a significant portion of the total (~70%). This digestion is accompanied by a distinct increase in mobility as well as a more heterogenous migration pattern on SDS-PAGE for both murine gp71 and avian gp85. No evidence of protein degradation was found after glycosidase treatment of gp71 or of a nonglycosylated protein of the same virus, p30.

We conclude from the serological studies reported that the carbohydrate portion of gp71, as detected by periodic acid-Schiff staining or by labeling with glucosamine, is not necessary for reaction with antibody in radioimmunoprecipi-

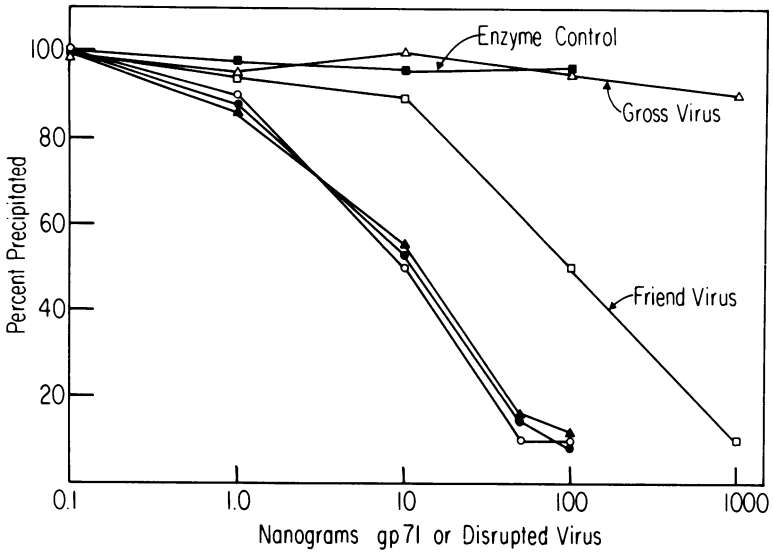

FIG. 7. *Type-specific precipitation of gp71. Rabbit antiserum to FLV gp71 absorbed exhaustively with STU cells producing Gross leukemia virus was reacted with FLV gp71 in direct radioimmunoassay. A limiting dilution of the serum (1/250 compared to 1/10,000 for the unabsorbed serum), precipitating 50% of iodinated FLV gp71, was used in the competition radioimmunoassay shown above. The type specificity of the serum is shown by the inability of disrupted Gross leukemia virus (△) to compete for FLV gp71 antiserum in contrast to disrupted FLV (□). Competition with untreated gp71 (○) and with gp71 treated with the glycosidase mixture (●) or neuraminidase (▲) is essentially indistinguishable. The enzyme controls (neuraminidase and glycosidase mixture) did not affect the precipitation of gp71 by the serum (■).*

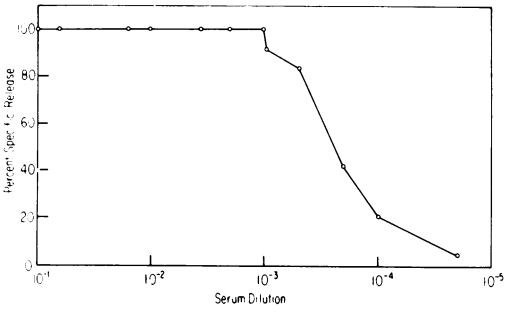

FIG. 8. *Titration of goat anti-FLV gp71 antiserum versus Eveline cells using the [^{14}C]NA release assay. See text for description of the microcytotoxicity assay. Results are expressed as percentage of specific release based on control of medium plus C'.*

tation, competition radioimmunoassay, or serum-mediated cytotoxicity analyses. On the other hand, treatment with proteolytic enzymes abolished all of the measurable serological reactivities of gp71, indicating that these specificities require the integrity of the protein portion of the molecule. In support of this conclusion it could be shown that poorly glycosylated intracellular precursors to avian gp85 were efficiently immunoprecipitated with antisera prepared against fully glycosylated gp85 (15).

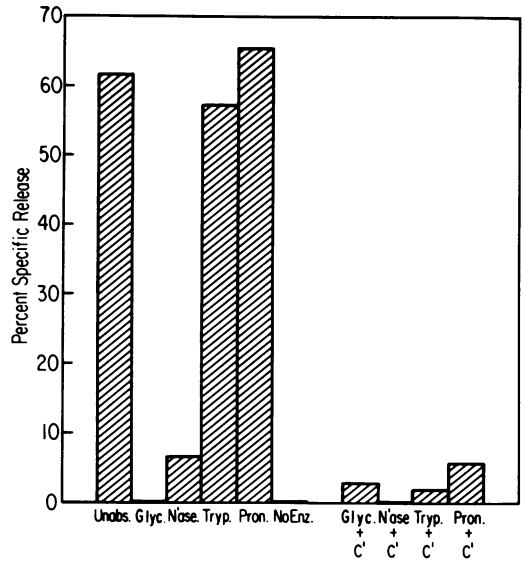

FIG. 9. *Effect of enzyme treatment on the ability of FLV gp71 to block the killing of Eveline target cells by goat anti-gp71 antiserum. Serum was used at a dilution of 1/500. Enzyme treatments and serum absorptions were carried out as described. Results are expressed as the percentage of specific release based on control of medium plus C'. Also included are controls of enzyme preparations plus C' which demonstrate that the enzymes themselves are not cytotoxic for the Eveline target cells.*

These results, however, do not necessarily imply that carbohydrate plays no role whatsoever in determining the immunological properties of gp71. Not all of the carbohydrate was removed by the glycosidase enzyme mixture, and the remainder might play a role in maintaining the proper configuration of the molecule. This is particularly true since the antisera used were raised against fully glycosylated gp71, and although they appear to react primarily with nonglycosylated portions of the molecule, their specificity probably depends on molecular conformation which could be influenced, at least to some degree, by the carbohydrate.

That the sugar portion of glycoprotein molecules can be responsible for determining the specificity of surface antigens on mammalian cells is well established, especially in the case of the human blood group antigens (19, 31). This appears to be true as well for at least some tumor-associated antigens, including the carcinoembryonic antigen of the human digestive system (3, 27). However, with the most carefully studied mammalian glycoprotein membrane antigens, namely those belonging to the mouse H-2 and human HL-A histocompatibility systems, it appears that the sugars do not play a significant role in conferring immunologic specificity, although this has not yet been conclusively established. Whereas early studies based on periodate treatment suggested that the carbohydrate portion was responsible for antigenicity (5, 8), more recent evidence, including a variety of general and highly specific protein-denaturing treatments and comparative studies of amino acid sequence between molecules of different haplotypes, strongly supports the notion that the protein portion of these molecules is the site of antigenic activity (23, 24).

On the other hand, membrane sugars can strongly influence the expression of surface antigens, both by imparting conformational restraints on membrane components and by their ability to "mask" subterminal membrane antigens. This has been consistently shown in a number of systems by the ability to enhance the immunogenicity of cells subsequent to treatment with terminal glycosidases such as neuraminidase (12). It has also been suggested that at least some virus-induced host-coded surface antigens could appear in transformed cells by a mechanism involving interference with the completion of glycoprotein sugar side chains allowing exposure of antigenic sites previously present but normally covered (10). In addition, Warren and his associates have demonstrated the presence of altered membrane glycoproteins in virus-transformed cells which contain additional fucose and/or neuraminic acid residues (32, 33). This may be related to the finding that the glycoprotein of oncornaviruses budding from transformed cells are distinguishable from those of particles released from nontransformed cells on the basis of their larger size (18).

The ability to remove carbohydrate without affecting the protein portion of the molecule will be helpful in determining various properties of viral glycoproteins, including their chemistry and structure. More detailed studies are in progress to determine the extent of carbohydrate removal, as well as whether distal or internal sugars are preferentially removed. The role of the carbohydrate portion of gp71 in other biological or immunological functions such as virus infectivity, attachment to cell surfaces, hemagglutination, and recognition by the host immune system are now accessible to study.

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