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Biochemical and Immunological Characterization of the Major Envelope Glycoprotein of Bovine Leukemia Virus

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The major envelope glycoprotein of bovine leukemia virus was isolated by lectin-bound Sepharose and DEAE-cellulose column chromatography. This protein was shown to have a molecular weight of about 51,000 and to lack detectable immunological cross-reactivity with glycoproteins of other oncornaviruses. Sera obtained from 100% of cattle examined with clinically diagnosed lymphosarcoma contained high-titered antibody to ^{125}I-labeled bovine leukemia virus glycoprotein, whereas sera from animals in a disease-free herd were antibody negative.

Lymphosarcoma of domestic cattle provides a unique model system for studies on the role of oncornaviruses in tumors of their natural hosts. The etiological association of this disease with a horizontally transmissible infectious agent, bovine leukemia virus (BLV), is well established (2, 14). Previously, we described the development of a radioimmunoassay for the major 24,000-molecular-weight (p24) structural protein of BLV (4). Application of this radioimmunological technique to studies of the epidemiology of bovine lymphosarcoma led to the demonstration of high-titer antibodies to BLV p24 in sera of 100% of animals with clinically diagnosed lymphosarcoma (4). In a comparison of presently available serological tests for detection of antibodies to BLV, radioimmunoprecipitation of ^{125}I-labeled BLV p24 was shown to be much more sensitive than previously described procedures (3). A number of sera, however, were observed that exhibited positive reactivity by agar gel immunodiffusion analysis but failed to precipitate ^{125}I-labeled BLV p24 to a significant extent (3). One possible explanation for these findings was that the positive reactivity detected by agar gel immunodiffusion might be attributable to antibodies directed against the envelope proteins of BLV.

There is accumulating evidence that oncornaviruses possess high-molecular-weight glycosylated proteins as constituents of their envelopes. As a consequence of their location on the surface of the virion, these glycoproteins have a major role in elucidation of host immune responses to exogenous virus infection. Moreover, immunoglobulins directed against viral envelope glycoproteins have been shown to neutralize viral infectivity (7, 9, 10, 17). The isolation and immunological characterization of envelope glycoproteins of various type C and type D RNA viruses have revealed these proteins to be of about 70,000 in molecular weight and to exhibit type-, group-, and interspecies-specific antigenic determinants (1, 7, 19). Whereas the immunological properties of type B oncornavirus glycoproteins have been less well characterized, the molecular weight of the major envelope glycoprotein of a representative virus of this group, mouse mammary tumor virus (MMTV), has been shown to be about 52,000 (5, 13, 15, 16).

Whereas the purification and development of competition immunoassays for glycoproteins of several oncornaviruses have been achieved (7-9, 18, 19), this has been somewhat difficult because of the labile nature of these proteins. In the present study an improved technique for isolation of oncornavirus glycoproteins was developed based upon a combination of affinity column chromatography, using lectin-bound Sepharose (LH-Sepharose), and DEAE-cellulose chromatography. A 51,000-molecular-weight envelope glycoprotein of BLV isolated by this means was labeled at high specific activity with ^{125}I, using the recently developed iodogen reagent, and utilized for the development of a radioimmunoprecipitation assay.

BLV was concentrated from tissue culture fluids of chronically infected fetal lamb kidney cells by density gradient centrifugation. The virus (8 to 10 mg of protein) was disrupted by sonic treatment for 20 s in 0.05 M Tris-hydrochloride (pH 7.8) buffer containing 1.0% Triton X-100, clarified by centrifugation at 100,000 × *g*, and applied to an LH-Sepharose column (1.5 by 5 cm) prepared by the following procedure. Activated CH-Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) was washed with 1 mM HCl followed by 0.1 M α-methyl-glucopyranoside and 0.1 M sodium bicarbonate

(pH 8.3). A 20-mg amount of lectin from *Lens culinaris* (Boehringer Mannheim Biochemicals, Indianpolis, Ind.) was added to 10 g of activated Sepharose in 50 ml of 0.1 M α-methylglycopyranoside, and the mixture was gently stirred for 4 h at 4°C. Excess lectin was removed by washing with 0.1 M α-methylglucopyranoside, and the remaining active groups were blocked with 5 mM ethanolamine, pH 8.4. The LH-Sepharose was washed with three cycles of alternating pH, using 0.1 M sodium bicarbonate (pH 8.3) and 0.1 M sodium acetate (pH 4.0), and stored in 0.01 M sodium phosphate (pH 7.4)-0.15 M NaCl buffer. The column was washed with phosphate-buffered saline at 4°C until free of absorbance at 280 nm, and the bound glycoprotein was eluted with 0.1 M α-methylmannopyranoside at room temperature. Fractions containing a 51,000-molecular-weight protein, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12), were pooled, dialyzed against TET (10 mM Tris (pH 7.8), 0.2 mM EDTA, 0.5% Triton X-100) buffer, and applied to a DEAE-cellulose (Whatman, H. Reeve Angle and Co., Clifton, N.J.) column (1.5 by 5 cm) equilibrated with the same buffer. The column was washed with TET buffer, and bound protein was eluted with a linear gradient of 0.0 to 0.5 M NaCl. Fractions containing a single protein of about 51,000 in molecular weight (BLV gp51) were pooled, aliquoted, and stored under liquid nitrogen.

Following purification, BLV gp51 was labeled with ^{125}I at high specific activity (5 to 20 μCi/μg) by a newly developed procedure utilizing the iodogen reagent (Pierce Chemical Co., Rockford, Ill.). For this purpose 10 mg of iodogen was dissolved in 1 ml of chloroform and aliquoted (20 μl) into several vials. After removal of chloroform by evaporation, 10 to 15 μg of BLV gp51 in 0.05 ml of TET buffer and 1 mCi of ^{125}I (Amersham/Searle, Arlington Heights, Ill.) were added. The reaction mixture was allowed to stand at room temperature for 60 s, and the iodinated glycoprotein was separated from free ^{125}I by P-10 (Bio-Rad Laboratories, Richmond, Calif.) column chromatography. Fractions containing ^{125}I-labeled glycoprotein were pooled, aliquoted, and stored at −20°C. A determination of radiochemical purity, as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, indicated that ^{125}I-labeled BLV gp51 migrated as a single radioactive peak with a molecular weight of about 51,000 relative to protein standards (Fig. 1).

Antisera produced in goats by active immunization with detergent-disrupted BLV as well as sera from cattle with clinically diagnosed lymphosarcoma were tested for immunoprecipitation of ^{125}I-labeled BLV gp51. As shown in Table 1, each of the anti-BLV sera tested bound ^{125}I-labeled BLV gp51 at high titer (<1:10,000) and, at lower serum dilution, to a final extent of over 90%. In contrast, high-titered antisera produced against each of several other oncornaviruses failed to bind ^{125}I-labeled BLV gp51. The specificity of immunoprecipitation of ^{125}I-labeled BLV gp51 was further demonstrated by the fact that sera with high antibody titers against BLV glycoprotein did not bind ^{125}I-labeled glycoproteins of representative type B, C, or D oncornaviruses to significant extents.

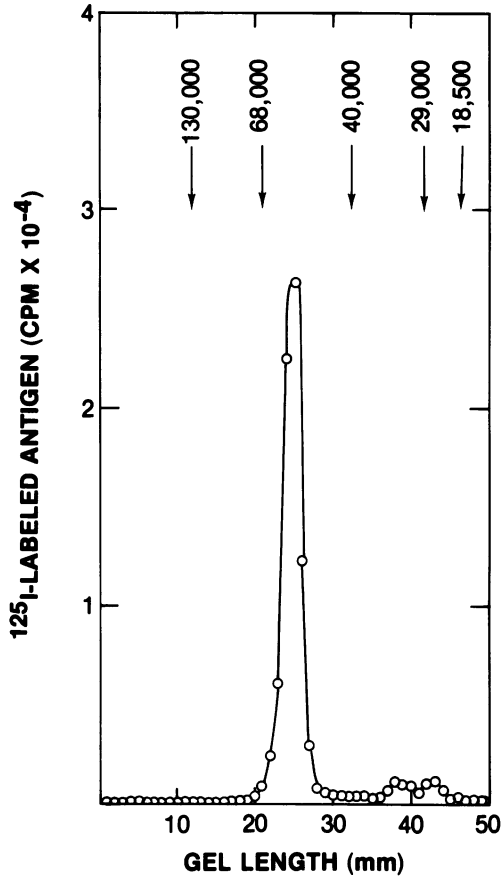

FIG. 1. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of ^{125}I-labeled BLV gp51. About 300,000 cpm ^{125}I-labeled BLV gp51 was subjected to electrophoresis on 55-mm sodium dodecyl sulfate-polyacrylamide gels (8.75%) at 2.5 mA per gel for 3 h. After electrophoresis, the samples were either stained with Coomassie blue or sliced into 1-mm fractions and tested for radioactivity in a Searle gamma counter model 1285. Molecular weight standards used for calibration included β-galactosidase (130,000), bovine serum albumin (69,000), aldolase (40,000), carbonic anhydrase (29,000), and β-lactoglobulin (18,500).*

TABLE 1. *Comparison of abilities of antisera to bind ^{125}I-labeled glycoproteins of representative oncornaviruses*

Serum[a]	Antiserum titer for binding ^{125}I-labeled envelope glycoproteins[b]				
	BLV (gp51)	MMTV (gp52)	Mason-Pfizer monkey virus (gp70)	Murine leukemia virus (gp70)	Baboon virus (gp70)
Antiserum to:					
BLV	25,600	<10	<10	<10	<10
MMTV	<10	21,200	<10	<10	<10
Mason-Pfizer monkey virus	<10	<10	450,000	<10	640
Murine leukemia virus	<10	<10	<10	10,600	<10
Baboon virus	<10	<10	<10	100	10,000
Bovine serum					
L-2	12,800	<10	<10	<10	<10
L-12	51,200	<10	<10	<10	<10
L-5	25,600	<10	<10	<10	<10

[a] Antisera to detergent-disrupted oncornaviruses were prepared in goats (20). Bovine sera were from cattle with clinically diagnosed lymphosarcoma.

[b] The envelope glycoproteins of Mason-Pfizer monkey virus, Rauscher murine leukemia virus, and *Papio cyanocephalus* baboon virus were isolated as previously described (7, 8, 18). The 52,000-molecular-weight envelope glycoprotein of MMTV (gp52) was isolated by a procedure similar to that used in the present study for BLV gp51 (Devare and Stephenson, unpublished results). Sera were assayed at serial twofold dilutions for binding ^{125}I-labeled glycoproteins in 0.2-ml reaction mixtures containing ^{125}I-labeled glycoprotein (approximately 10,000 cpm), 0.01 M Tris-hydrochloride (pH 7.8), 0.01 M EDTA, 0.4% Triton X-100, 1% bovine serum albumin, and 0.2 M NaCl and were incubated for 3 h at 37°C followed by 4°C for 18 h. After the addition of 0.025 ml of antiserum to immunoglobulin G to each tube to precipitate antigen-antibody complexes, reaction mixtures were incubated further for 1 h at 37°C and 3 h at 4°C. One milliliter of cold 10 mM Tris-hydrochloride, pH 7.8, and 10 mM NaCl buffer containing 0.1% Triton X-100 was added to each tube, samples were centrifuged for 15 min at 2,500 rpm, supernatants were aspirated, and the radioactivity in the precipitates was determined. Serum titers are expressed as the reciprocals of the highest dilutions capable of binding 20% of the ^{125}I-labeled glycoproteins and represent mean values of three separate determinations.

Moreover, this reactivity could be fully competed by detergent-disrupted BLV, but not by other virus isolates tested, including feline leukemia virus, murine leukemia virus, baboon virus, woolly monkey virus, Mason-Pfizer monkey virus, or MMTV (Fig. 2).

To compare immunoprecipitation of ^{125}I-labeled BLV gp51 to the previously described radioimmunoassay for p24 (4) for studies of BLV infection in cattle, sera from a number of cattle from herds with known high incidences of bovine lymphosarcoma as well as sera from animals with clinically diagnosed lymphosarcoma were tested in both assays. The results indicate an excellent correlation between the titers for

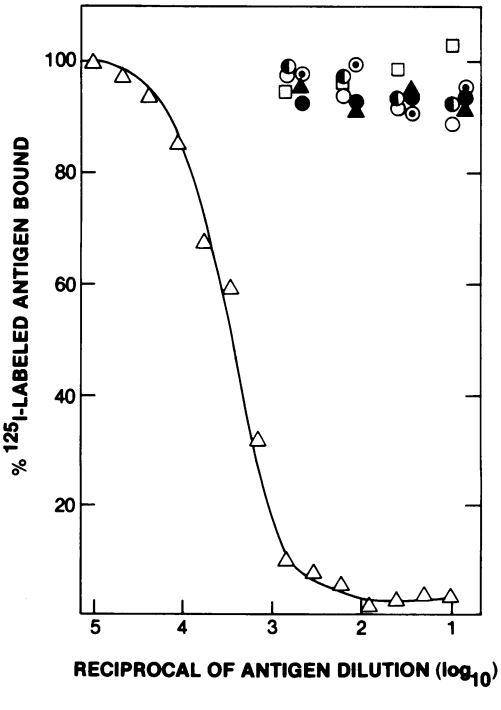

FIG. 2. *Specificity of immunoprecipitation of ^{125}I-labeled BLV gp51. Unlabeled detergent-disrupted viral antigens were tested at serial twofold dilutions for ability to complete with ^{125}I-labeled BLV gp51 for binding limiting amounts of antiserum to BLV. The assay conditions were as described in Table 1. Viruses tested included BLV (△), Rauscher murine leukemia virus (□), Rickard feline leukemia virus (▲), Papio cyanocephalus baboon virus (○), woolly monkey virus (⊙), Mason-Pfizer monkey virus (◐), and MMTV (●).*

precipitation of ^{125}I-labeled BLV gp51 and those observed for precipitation of p24 (Fig. 3). Moreover, whereas 100% of the animals tested were antibody positive, the actual titers were generally higher in sera of animals with confirmed lymphosarcoma. In contrast, sera from each of 20 animals from an isolated lymphosarcoma-free herd failed to precipitate either labeled antigen (data not shown). The present findings thus argue against the possibility that the positive reactivity observed by agar gel immunodiffusion in sera lacking detectable antibody to BLV p24 sera could be attributable to antibody directed against BLV glycoprotein.

The viral origin of the 51,000-molecular-weight protein isolated in the present study was demonstrated by the finding that its binding by both natural and immune sera could be fully competed by extracts of BLV-infected fetal lamb kidney cells but not by extracts of uninfected control cultures (data not shown). BLV gp51 was shown to be glycosylated by its binding to lectin as well as its comigration on sodium dodecyl sulfate-polyacrylamide gel electrophoresis with the major [^{3}H]glucosamine-labeled BLV structural protein (unpublished observations). Whereas BLV gp51 was of lower molecular weight than known type C or type D RNA oncornavirus glycoproteins, its molecular weight closely resembled that of the major envelope glycoprotein of MMTV. Further similarity between BLV and MMTV is indicated by the fact that the molecular weight of the major nonglycosylated structural protein of both viruses is about 24,000 (5, 13, 15, 16). Moreover, BLV and MMTV can be distinguished from type C RNA viruses on the basis of the divalent cation preference of their RNA-dependent DNA polymerases (6, 11). On the basis of all of these considerations, BLV appears to closely resemble MMTV and should, therefore, be included with MMTV in the type B oncornavirus group.

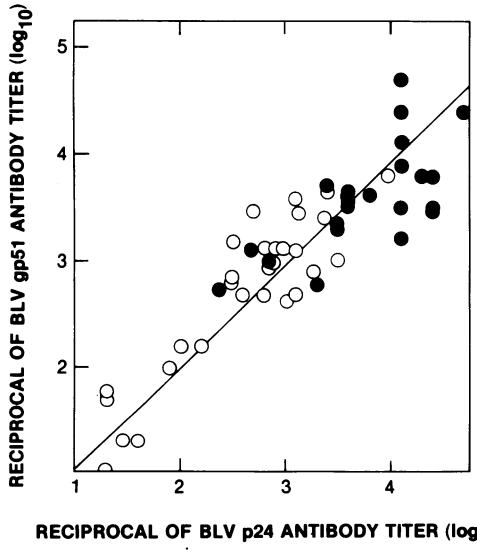

FIG. 3. *Comparison of immunoprecipitation tests for BLV p24 and gp51 for detection of antibodies in BLV-exposed clinically normal cattle (○) and cattle with histopathologically diagnosed lymphosarcoma (●). Serum titers are expressed as the reciprocals of the highest dilutions capable of binding 20% of the appropriate ^{125}I-labeled proteins and represent mean values of three separate determinations.*

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