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Characterization by capillary electrophoresis of the surface glycoproteins of ovine lentiviruses before and after treatment with glycosidic enzymes

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ABSTRACT

Ovine lentiviruses are a group of viruses that infect sheep and goats. These viruses contain a surface glycoprotein (SU) that is very similar among the viral strains. Sera from infected animals react equally well with SU from each strain. Monoclonal antibodies produced to SU can distinguish among some of the viral strains. In order to delineate these differences we treated SU from several viral strains with the glycosidic enzymes. These enzymes included a mixture of exoglycosidases, β-N-acetyl glucosaminidase, neuraminidase and endoglycosidases D, F and H. After these treatments we observed changes in the reactivities of the monoclonal antibodies that were directed to SU. In order to characterize these changes on the surface epitopes, SU from the different viral strains were subjected to free zone capillary electrophoresis (CZE) using an 0.02 *M* phosphate buffer at pH 9.0 at a running voltage of 5 kV. Differences were readily seen between SU that had not been treated and SU that had been treated with the glycosidic enzymes. Each viral strain had a characteristic electropherogram. The electropherograms indicated that the heterogeneity of the charge on SU was increased after the enzyme treatments. From these results we have concluded that the carbohydrate moieties play an important role in contributing to the surface charge of SU. This charge affects the nature of its surface epitopes and has an impact on its biological function.

INTRODUCTION

Ovine lentiviruses belong to a family of retroviruses that infect sheep and goats and cause disease affecting multiple systems. The prototype virus, maedi-visna, was first isolated in Iceland in the 1950s [1,2]. Since this time, several additional strains of ovine progressive pneumonia virus (OPPV) have been isolated [3–5]. In cell culture these strains show characteristic differences [6–9]. Except for the nucleic acid sequence of a few of these viruses [10–12], structural properties that may cause these strain differences have not be explored. Advances in the adaptation of high-performance capillary electrophoresis for proteins point to this technique as an efficient analytical tool to observe differences in protein structures [13–16]. We chose this technique to observe changes that occur on the surface glycoprotein (SU) of ovine lentiviruses, after treatment with glycosidic enzymes. This glycoprotein plays an important role in the interaction of the host cell with the virus. Recently, the role that carbohydrate moieties play in immunogenicity and pathogenicity for lentiviruses in general (this family includes the human immunodeficiency virus, HIV) has taken on more significance. Hansen *et al.* [17] have shown that peripheral glycosylation of the surface glycoprotein of HIV may be a target for virus neutralization and, more recently, that monoclonal antibodies to carbohydrate epitopes of gp120 of HIV inhibit infection of lymphocytes [18]. Benjouad *et al.* [19] have suggested that carbohydrate moieties can modulate the specificity of the antibody response to the important V3 loop region of HIV. In other experiments, cell specific

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glycosylation determined whether a strain of feline immunodeficiency virus was cytopathic or non-cytopathic [20]. In earlier work on caprine arthritis encephalitis virus (CAEV) Huso *et al.* [21] found that sialylation of CAEV made it resistant to degradation by proteolytic enzymes. The cross-reactivity that has been shown among lentiviruses, specifically equine infectious anemia virus reactivity with HIV antisera, is due to reaction with similar carbohydrate moieties [22]. We have found that treatment of SU from the ovine lentiviruses with glycosidic enzymes will reduce or cause a loss of the reactivity for some of the monoclonal antibodies produced to SU. In this study, we investigated differences in glycoproteins from different viral strains and changes both in antigenicity and in the electrophoretic mobility of SU after treatment with glycosidic enzymes.

MATERIALS AND METHODS[a]

Purification of the surface glycoprotein (SU)

Ovine fetal lung cells that had been persistently infected with a strain of an ovine lentivirus were grown for 2 weeks in Eagles's minimal media containing 10% ovine serum. The ovine lentiviral strains in this study were WLC-1, Visna, Howard and Runk. A caprine lentivirus, CAEV was also included. Each of the strains was cultured as above and then processed in the same manner. Culture fluids were harvested and precipitated with 50% saturated ammonium sulfate. The precipitates were harvested by centrifugation, resuspended, and dialyzed against 0.05 *M* sodium phosphate containing 0.15 *M* NaCl, pH 7.0 (phosphate-buffered saline, PBS). After dialysis, the concentrated culture fluids were placed over a lentil lectin Sepharose 4B column (Pharmacia LKB Biotechnology, Uppsala, Sweden). The column was washed with PBS and the glycoproteins eluted with 0.5 *M* α-D-methylmannoside in PBS. The eluted glycoproteins were precipitated with 50% saturated ammonium sulfate. The samples were dialyzed against PBS and placed on a protein G-Sepharose column to remove contaminating immunoglobulins. Chromatography was performed using the methods specified by the manufacturer (Pharmacia LKB Biotechnology). SU did not bind to this column. The unbound fractions were concentrated as above. When SU is prepared in this way, one diffuse band is observed on sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Enzyme treatments

The protein concentration of each of the glycoproteins from the different strains was determined [23] and made equivalent to 1.92 mg/ml. SU was dialyzed against 0.2 *M* phosphate–citrate buffer pH 5.5. After dialysis, SU was centrifuged to remove particulates. Exoglycosidases (*T. cornutus*, a mixture of several glycosidases, ICN, Costa Mesa, CA, USA) were used in a ratio of 1:1 (w/w) to SU at 37°C for 16 h. Neuraminidase was used in a ratio of 0.05 U/380 μg of glycoprotein and β-N-acetyl glucosaminidase, 0.06U/380 μg of glycoprotein. The above enzymes were obtained from Sigma (St. Louis, MO, USA). SU was incubated with each of these enzyme alones at 37°C for 16 h. In another experiment, SU was pretreated with neuraminidase for 4 h before the addition of the enzyme β-N-acetyl glucosaminidase. Those samples that were to be tested by radioimmunoassay were diluted 1/100 before enzymatic treatments. Those tested by capillary electrophoresis were not diluted further. When the detector was set at 214 nm *ca.* 60 ng were injected into the capillary. At 200 nm *ca.* 35 ng were injected into the capillary.

Capillary electrophoresis

Capillary electrophoresis was performed on a P/ACE system Model 2050 (Beckman, Fullerton, CA, USA) controlled by the System Gold software (Beckman). UV Absorbance was monitored by a fixed-wavelength detector at either 214 or 200 nm. The capillary was 57 cm long ×

[a] *Disclaimer:* No endorsements are herein implied. Brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standards of the products, and the use of the names by the USDA implies no approval of the products to the exclusion of others that may also be suitable.

75 μm I.D. Two buffers and two different voltages were tried to establish conditions for separation of the glycoprotein. The buffer systems that were used were: 0.02 *M* phosphate buffer at two different pH values, 9.0 and 7.0, and 0.1 *M* sodium borate pH 8.5. The separating voltages of 5 kV and 10 kV were used. The separating buffer that was selected was 0.02 *M* phosphate buffer, pH 9.0 with a separating voltage of 5 kV at 25°C. When the detector was set at 214 nm the sample was injected by pressure for 5 s and at 200 nm, for 3 s. Electropherograms were produced for the glycoproteins from each of five different strains of the virus and for each of the enzyme treatments of each viral glycoprotein.

Solid-phase radioimmunoassay

Monoclonal antibodies had been previously produced to the purified SU. Each monoclonal antibody was purified by Protein G-Sepharose chromatography using the methods specified by the manufacturer. (Pharmacia LKB Biotechnology). The purified monoclonal antibody was iodinated using I-o-do-Gen (Pierce, Rockford, IL, USA) [24]. The solid-phase radioimmunoassay used was a modification of the procedure of Srikumaran *et al.* [25]. Briefly, 100 μl (*ca.* 2 μg) of the samples including the enzyme treated glycoprotein was adhered to the plates at 37°C for 2 h. After incubation, the plates were washed five times with a buffer containing 4 m*M* KCl, 140 m*M* NaCl, 1.5 m*M* KH_2PO_4 and 6 m*M* Na_2HPO_4, pH 7.3 and 1.0% bovine serum albumin. Approximately 100 000 cpm of the ^{125}I monoclonal antibody were added to the plate. The plate was incubated at 37°C for 2 h and then at 4°C overnight. The plates were washed as above, and the individual wells cut out and counted in a gamma counter. Two representative monoclonal antibodies, were chosen for this assay, one (9H4) that reacts specifically with the viral strains WLC-1, Visna and CAEV and another (10C10) that reacts with Howard and Runk. After subtracting the background cpm from both the sample cpm and total cpm the loss of activity and the % enhancement were calculated as follows:

$$\% \text{ loss of activity} = 100 \times \frac{\text{total cpm bound} - \text{sample cpm}}{\text{total cpm bound}}$$

$$\% \text{ enhancement} = 100 \times \frac{\text{sample cpm}}{\text{total cpm bound}}$$

The error in this assay was ±7.0%. The results obtained in these assays have been reproduced at least three times.

RESULTS

Profiles of SU run with phosphate buffer and borate buffer at 10 kV are shown in Fig. 1a. A lower voltage of 5 kV was used with these buffers (Fig. 1b). The wavelength at 200 nm was used with the phosphate buffer system at this voltage as well (Fig. 1b). At this wavelength (200 nm) an increase in sensitivity of *ca.* 2.5-fold was observed. These profiles were reproducible from capillary to capillary and from different preparations of SU.

Profiles of the enzymes, neuraminidase and β-N-acetyl glucosaminidase, that were used to treat the glycoproteins are shown in Fig. 2a and b. Little or no absorbance was observed for

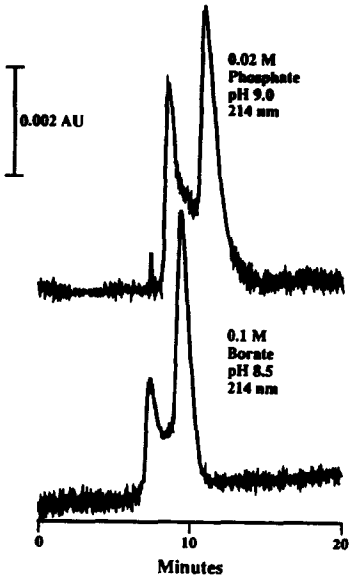

Fig. 1.

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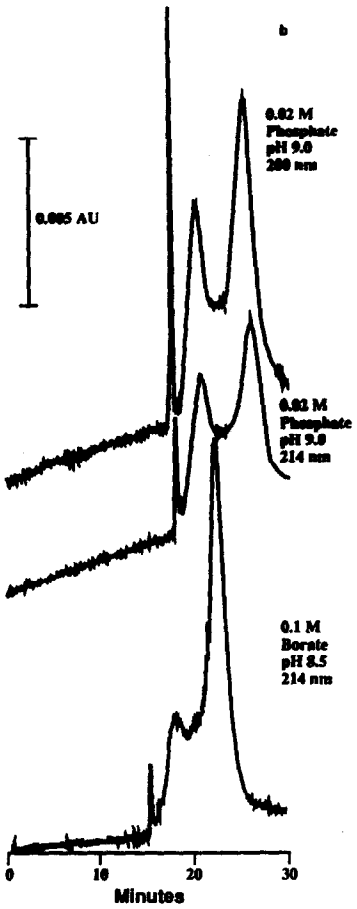

Fig. 1. Electropherograms of different conditions for SU from the strain of virus WLC-1. A Beckmann P/ACE was used. (a) 0.02 *M* sodium phosphate buffer, pH 9.0 and 0.1 *M* sodium borate buffer, pH 8.5 at 10 kV at 214 nm; (b) 0.02 *M* sodium phosphate buffer at 214 nm and 200 nm at 5 kV; 0.1 *M* sodium borate buffer, pH 8.5 at 214 nm at 5 kV.

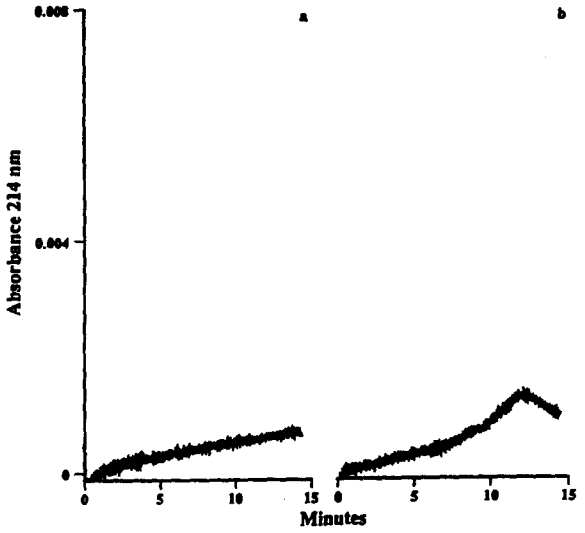

Fig. 2. Electropherograms of the enzymes neuraminidase and β-N-actyl glucosaminidase. The capillary was 57 cm × 75 μm I.D. and the separating voltage was 20.5 kV. UV absorbance was measured at 214 nm. The sample loaded into the capillary was 30 nl. A Beckman P/ACE was used. (a) β-N-Acetyl glucosaminidase; (b) neuraminidase.

these enzymes at the concentrations used to treat the glycoproteins. Profiles were obtained of SU from different viral strains after treatment with the mixed exoglycosidases. The exoglycosidases were used at a concentration equivalent to that of SU. The absorbance profile of the exoglycosidases alone was close to that of SU making the profiles of the enzyme treated samples difficult to interpret (data not shown). The exoglycosidases did cause a loss of antigenic activity of SU with the monoclonal antibodies (Table I). Removal of the sialic residues by neuraminidase appears to affect the profile of the strains by changing the mobility and the number of peaks in the electropherogram (Fig. 3b). Although the removal of these residues had little effect on the antigenicity of Howard or WLC-1,

TABLE I

LOSS OF ACTIVITY AFTER TREATMENT WITH EXOGLYCOSIDASES [%]

Monoclonal antibody	Virus strain				
	Runk	Howard	WLC-1	CAEV	Visna
10C10	77.5	89.9	–	–	–
9H4	–	–	75.7	47.7	59.5

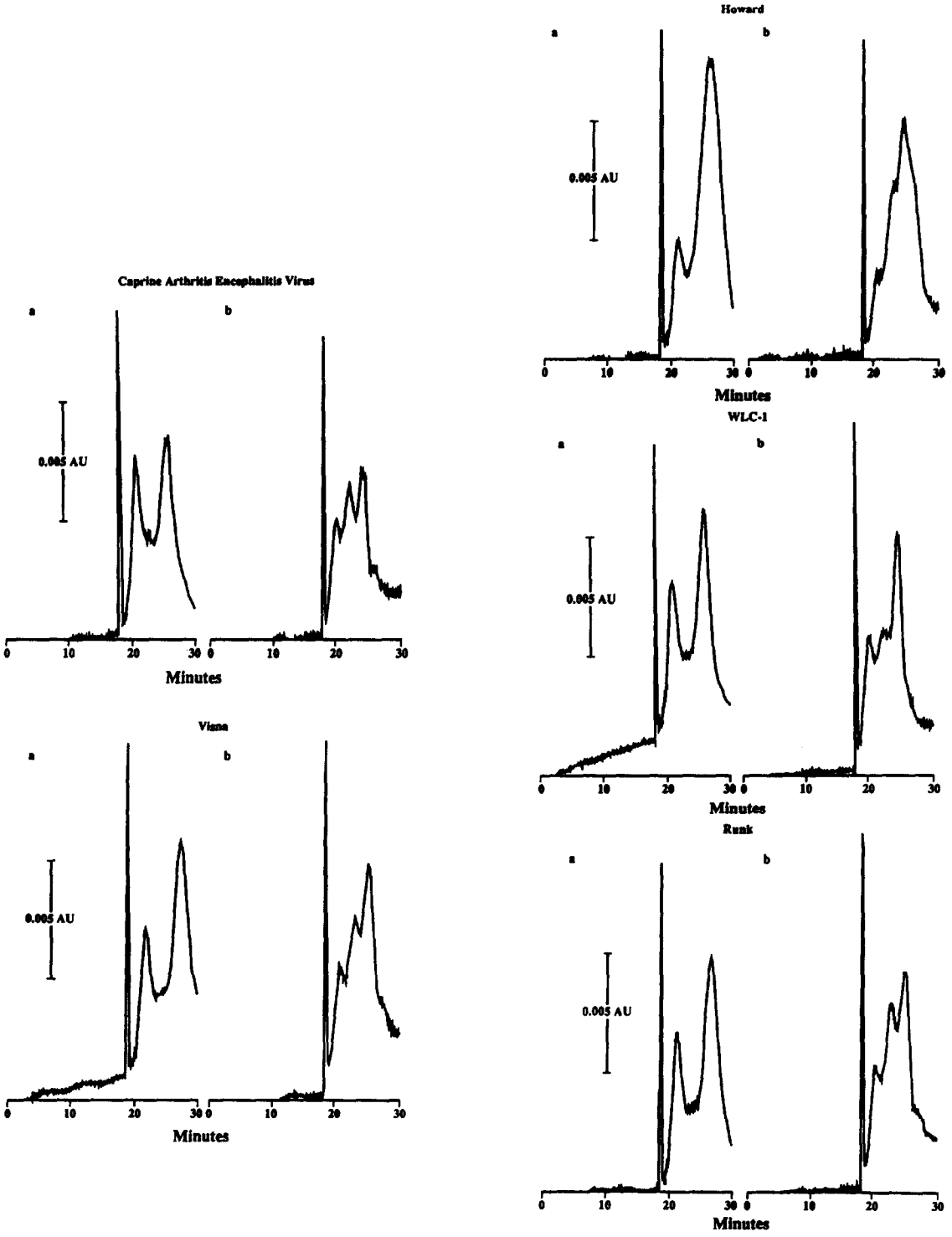

Fig. 3. Electropherograms of SU before and after treatment with neuraminidase. A Beckman P/ACE was used. The capillary was 57 cm × 75 μm I.D. and the separating voltage was 5 kV. UV absorbance was measured at 200 nm. The sample loaded into the capillary was 18 nl. The virus strain is indicated on the top of each panel. (a) The untreated glycoprotein; (b) the glycoprotein treated with neuramidase.

TABLE II

ENHANCEMENT OF ACTIVITY AFTER TREATMENT WITH NEURAMINIDASE [%]

Monoclonal antibody	Virus strain				
	Runk	Howard	WLC-1	CAEV	Visna
10C10	112.7	0	–	–	–
9H4	–	–	105.0	112.6	119.0

the antigenicity of Runk, Visna and CAEV was enhanced (Table II). The antigenicity of SU of the viral strain Howard does not appear to be affected by the enzymatic treatments. This is consistent with the different reactivities to this strain exhibited by a panel of monoclonal antibodies produced to the glycoprotein (unpublished data).

The enzymes, β-N-acetyl glucosaminidase and the endoglycosidases (data not shown), had little or no effect on any of the strains for capillary electrophoresis or for antigenicity. Prior treatment with neuraminidase followed by β-N-acetyl glucosaminidase did not alter the profile from that obtained with neuraminidase alone (Fig. 3b).

The monoclonal antibody 9H4 reacts poorly with the strains Runk and Howard while it reacts very well with WLC-1, Visna and CAEV. The monoclonal antibody 10C10 reacts in an opposite fashion. Treatment of the glycoproteins with neuraminidase enhanced the reactivity to the glycoproteins by the monoclonal antibody (9H4) for Visna and CAEV (Table I) and by the monoclonal antibody 10C10 for Runk (Table II). Exoglycosidases reduced the reactivity for all the glycoproteins between *ca.* 50% and 90%.

DISCUSSION

Capillary electrophoresis can be used to analyze significant changes that occur in biological systems. There has been considerable effort to define conditions for protein separation and for prevention of protein interaction with the capillary walls [26–29]. We used a simple phosphate buffer system without additives. We tried several ionic detergents and found that in some cases, the detergents caused negative peaks and interfered with the ultraviolet absorbance at the wavelengths that we wished to use. Landers *et al.* [28] have suggested that borate, itself, was a necessary component to obtain optimal resolution in the separation of glycoforms of ovalbumin. With SU borate buffer did not enhance the separation and the electropherograms were similar to those profiles obtained in the phosphate buffer system. Although we did not use buffer additives, perhaps a buffer additive such as putrescine as suggested by both Taverna *et al.* [30] and Landers *et al.* [28] would help to observe the microheterogeniety of glycoproteins based on their sialic acid content. When the method of Tran *et al.* [31] was followed in which the capillary was pre-equilibrated with the phosphate buffer at pH 4.0 several hours prior to obtaining the electropherogram, no peaks were obtained.

Capillary electrophoresis has contributed information that helps unravel the complex picture that affects the antigenicity of surface glycoproteins of strains of ovine lentiviruses after treatment with different glycosidic enzymes. Charge differences can explain the differences in reactivities to some of the monoclonal antibodies. Epitopes that are hidden by the sialic acids are exposed after treatment with neuraminidase and migration patterns are changed on the electropherograms. Sialic acids were noted to be important for the viral strain CAEV. Huso *et al.* [21] reported that the sialic acids conferred a degree of resistance to degradation of the virus by proteolytic enzymes and to neutralization of the agent by antibodies. He concluded that the sialylation pattern of the CAEV envelope is responsible for its poor antigenicity and inability

to induce neutralizing antibodies. He found that another strain, Visna, which has fewer sialic acids on its surface is an excellent inducer of neutralization antibodies. Our finding that the monoclonal antibodies' reactivity is enhanced by removal of the sialic acids agrees with these conclusions. Although the strain of CAEV used is not identical to the one used by Huso *et al.* [21], we also found an increase in the reactivity of monoclonal antibodies to CAEV after treatment with neuraminidase. This suggests that the carbohydrate configuration plays a role in biological function of these viruses as well as determining the antigenicity of SU. We can speculate that the host (sheep *vs.* goats) pattern of glycosylation of SU of this virus may help determine the course of pathogenicity and virulence of the virus. Although the correlation of virulence with glycoslyation was not studied, it is known that some strains of the virus cause clinical disease more readily than others do. The changes induced by the glycosidic enzyme treatments in the reactivity of the monoclonal antibodies to the glycoproteins of different strains and the profiles on capillary electrophoresis have demonstrated that the glycosylation patterns contribute directly to the antigenicity of SU and the surface charge of SU as determined by capillary electrophoresis.

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