

Purification of a Glycoprotein from Bovine Leukemia Virus (BLV)

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A precipitating antigen of bovine leukemia virus was isolated by isoelectric focusing and Sephadex gel filtration. In SDS-polyacrylamide gel electrophoresis it was found to be a homogeneous protein with a relative molecular weight of 69 000 daltons. Because of its relative molecular weight and staining characteristics it was designated as BLV gp69. A protein with the same molecular weight could also be demonstrated in BLV particles. In 34 out of 35 sera from cattle affected by enzootic bovine leukosis antibodies against gp69 were detected, whereas the sera from 197 animals, free of bovine leukosis, did not react in immunodiffusion test.

The morphological and biological properties of the putative aetiological agent of enzootic bovine leukosis are similar to those of other mammalian oncornaviruses. The virus particles consist of a central electron-dense core and an envelope which possesses knob-like structures¹. They also contain a high molecular weight RNA of 60–70 s², and an

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RNA-directed DNA polymerase³. In murine leukemia virus, the main constituent of the knobs is a glycoprotein with a molecular weight of 71 000 daltons⁴. This major glycoprotein has type-specific as well as species-specific and interspecies-specific antigenic determinants⁵.

To study the biological properties of a glycoprotein of BLV attempts were made to isolate it from purified virus particles. The supernatant from fetal lamb kidney cells infected with BLV⁶ was centrifuged for 90 min at 18 000 rpm (rotor 19, Spinco ultracentrifuge) and the resulting concentrated virus pellet was suspended overnight in phosphate buffered saline (PBS). Then, the suspension was clarified by low-speed centrifugation (20 min, 6000 rpm), layered on a discontinuous sucrose gradient (20/35/50%) and centrifuged for 60 min at 26 000 rpm (rotor SW27). The virus found in the region of 1.16 g/cm³, was collected, diluted 1:2 in PBS, and sedimented at 26 000 rpm for 45 min (rotor SW27). Virus concentrated by these procedures was disrupted by the addition of 6 M guanidinium hydrochloride dissolved in TEN (0.01 M Tris HCl, pH 7.5, 0.01 M EDTA, 0.15 M NaCl) and the disrupted material was clarified by centrifugation at 32 000 rpm for 2 hours (rotor SW50). After dialysis of the supernatant against an aqueous solution of 1% glycine, 1% glycerol, 0.1% Triton X-100 and 0.1% Ampholine pH 3.5–10, a precipitating antigen was isolated by isoelectric focusing (IEF). Serial fractions of 2 ml were collected from the IEF-column

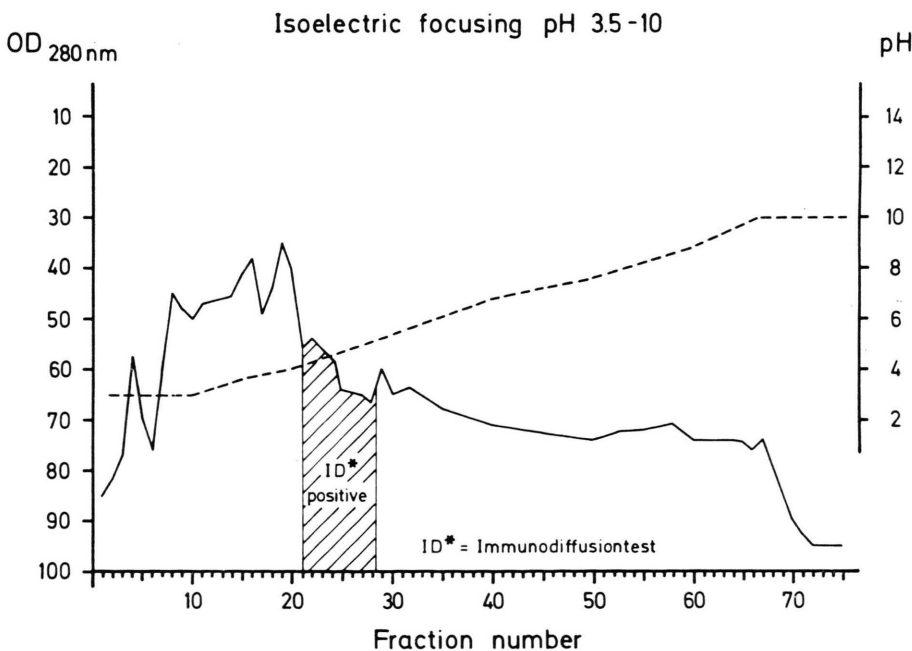


Fig. 1.
IEF was carried out in a sucrose gradient from 5–40% w/v, containing 3% Ampholine, pH 3.5–10, and an initial power of 4 watts. After 24 hours serial fractions of 2 ml were collected and screened by ID-tests.
(—) OD_{280 nm},
(---) pH gradient.



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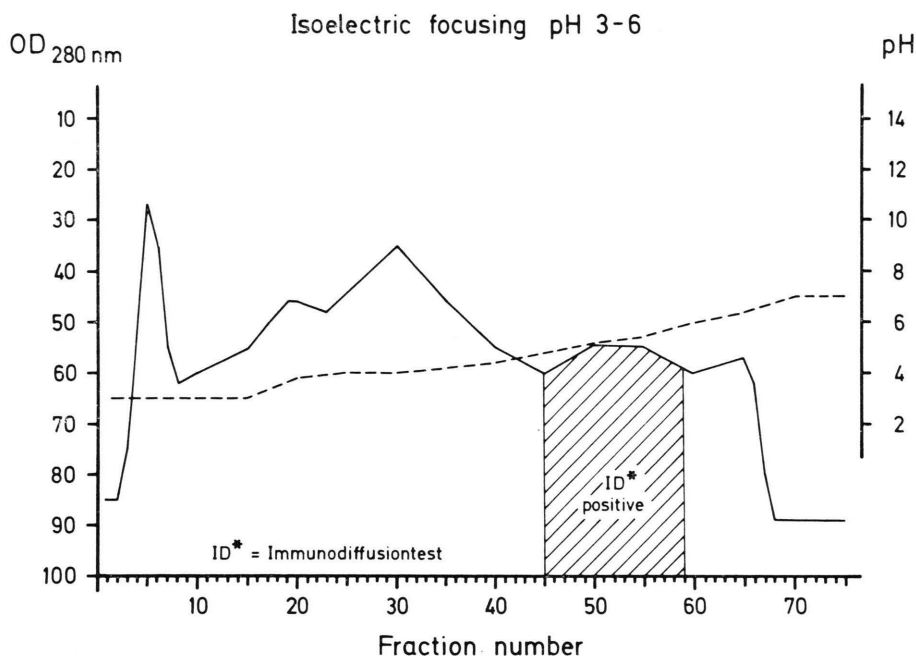


Fig. 2.
IEF performed
as described
in Fig. 1 using
Ampholine with a
pH range from
3–6.

and were screened by agar gel immunodiffusion test (ID-test) with a serum from a cow suffering from bovine leukosis (Fig. 1). Serologically positive fractions were pooled and clarified by ultracentrifugation at 20 000 rpm for 20 min (rotor SW41). After addition of ammonium sulfate to a final concentration of 30% w/v the sample was again centrifuged at 20 000 rpm for 20 min (rotor SW41). The pellicle obtained by these procedures contained serological active material and was dissolved in IEF-buffer and further purified by a second isoelectric focusing step in a pH range from 3.0–6.0 (Fig. 2). The serologically active fractions were pooled, and treated as mentioned after the first IEF, *i.e.* ultracentrifugation, and precipitation by ammonium sulfate. The resulting pellicle was dissolved in sample buffer (Tris/HCl, pH 7.2, 0.001 M EDTA, 0.65 M NaCl, 1% Triton X-100) and finally purified by gel filtration on a Sephadex G 150 superfine column (1.5 × 90 cm) equilibrated with sample buffer without Triton X-100. The sample volume never exceeded 1 ml and the column was run with a flow rate of 2 ml/hour. Fractions of 2 ml were collected and screened for serological activity. The serologically active fractions were analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in 10% acrylamide:bisacrylamide gels using the discontinuous buffer system of Laemmli⁷. In each SDS-PAGE experiment marker proteins (bovine albumine, ovalbumine, and myoglobine or cytochrome C) were included. Stain-

ing with Coomassie brilliant blue showed that the antigen after chromatography on Sephadex G 150 consisted of an electrophoretically homogeneous protein with a relative molecular weight of 69 000 daltons (Fig. 3 a). Staining with periodic acid-Schiff (PAS) reagent indicated that the antigen contained carbohydrates (not shown). Analysis of BLV purified by sucrose gradient centrifugation, in SDS-PAGE showed that the virus contained a protein of the same molecular weight (Fig. 3 b).

The isolated antigen was therefore designated as BLV gp 69. In the ID-test a precipitation line was observed between purified gp 69 and a serum from a leukotic cow but no line appeared with an antiserum against bovine serum produced in a rabbit (Fig. 4). Furthermore, gp 69 obtained after the second IEF step showed a precipitation line of identity with a crude antigen received from Dr. J. M. van der Maaten, Ames, Iowa, USA, and considered to contain a glycoprotein from BLV (van der Maaten, personal communication) (Fig. 5). In addition, a correlation between the incidence of antibodies against gp 69 and the appearance of the enzootic bovine leukosis could be demonstrated by ID-tests with bovine sera from different farms. Thirty-five cattle sera from 6 different farms with a positive haematological record were tested for specific antibodies against BLV gp 69. Thirty-four of these sera were positive in ID-test with the gp-antigen, whereas the sera from 197 animals with normal

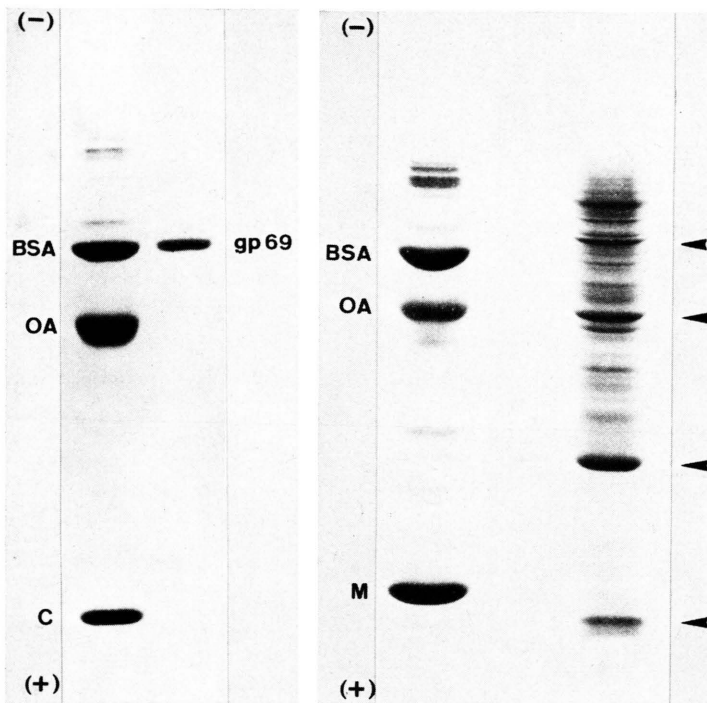


Fig. 3 a.

Fig. 3 b.

Fig. 3. a) SDS-PAGE of isolated glycoprotein (gp69) and marker protein (BSA, bovine serum albumine; OA, ovalbumine; C, cytochrome C) in 10% acrylamide: bisacrylamide slab gel. b) SDS-PAGE of BLV purified by sucrose gradient centrifugation and marker proteins (BSA, bovine serum albumine; OA, ovalbumine; M, myoglobine). Arrows indicate the main proteins of BLV.

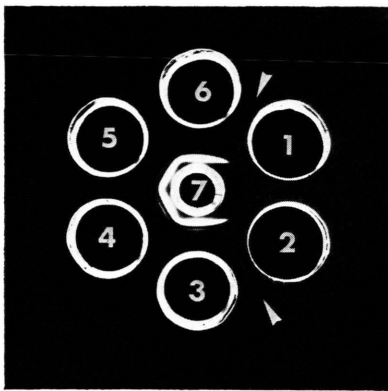


Fig. 4. Examination of purified glycoprotein in ID-test. Purified glycoprotein (1, 2), serum from a cow suffering from bovine leukosis (3, 4, 5, 6) and antiserum against bovine serum produced in a rabbit (7). Arrows indicate the precipitation lines between glycoprotein and bovine serum.

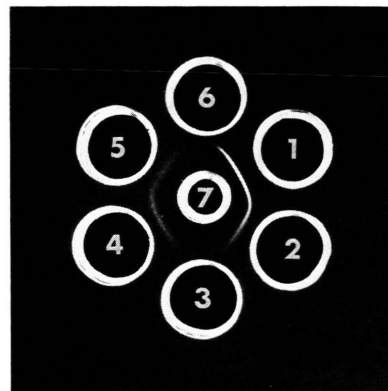


Fig. 5. ID-test with a precipitating antigen obtained from Dr. van der Maaten (3, 6), precipitating antigen from IEF (1, 2, 4, 5), and serum from a cow suffering from bovine leukosis (7), which was used in all ID-tests during the isolation procedure.

lymphocyte numbers, out of 98 farms free of bovine leukosis, did not react in the ID-test.

The described results show that a glycoprotein with a relative molecular weight of 69 000 daltons could be isolated from BLV. A high percentage of sera from animals affected by bovine leukosis reacted with this antigen in the ID-test. To study the biological properties of the purified gp in greater

details the production of a hyperimmune serum has been initiated.

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