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Note

High-performance gel-permeation chromatography of the surface glycoprotein from bovine leukemia virus

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Bovine leukemia virus (BLV) is a retrovirus that infects cattle and sheep. The virus consists of RNA, four non-glycosylated proteins (p24, p15, p12 and p10) and two glycosylated components (gp51 and gp30) [4]. The two glycoproteins have been characterized using monoclonal antibodies [2,3] and a partial amino acid sequence has been obtained [4,11] for these glycoproteins. One of these glycoproteins, the major surface glycoprotein (gp51) has been purified, iodinated and used for radioimmunoprecipitation [5]. Although traditional methods used in the purification of the gp51 preserved its antigenicity, the procedure was lengthy and yields were low. With the development of columns and methods for protein purification using high-performance liquid chromatography (HPLC) [1,6,12] an alternative purification procedure using a commercially available size gel-permeation column for the gp51 was made. The effects of a non-ionic detergent, Triton X100, and α-D-methylmannoside on the chromatographic profile of the gp51 after iodination were studied. After chromatography, the level of the antigenic activity of the unlabelled gp51 and antibody binding of the [^{125}I] gp51 were assayed.

EXPERIMENTAL

High-performance gel-permeation chromatography (HPGPC)

An M6000 solvent delivery system equipped with a U6K injector and a Model 440 UV absorbance detector set at 254 nm (Waters Assoc., Millipore Division, Milford, MA, U.S.A.) was used in these studies. Two high-performance gel-permeation columns (TSK 3000, 30 cm × 7.5 cm I.D.; Beckman Instruments, Palo

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Alto, CA, U.S.A.) were joined together and were preceded by a guard column. HPGPC was carried out at ambient temperature with a flow-rate of 1 ml/min. The mobile phase, 0.1 M dipotassium hydrogenphosphate (pH 7.0) containing 0.2 M sodium chloride (buffer A), was filtered and degassed before use. Molecular mass markers (US Biochemicals, Cleveland, OH, U.S.A.) were chromatographed to obtain a standard curve. The effect of including 0.05% Triton X100 or a combination of 0.1 M α--D-methylmannoside and the Triton X100 in the buffer was examined. After chromatography of [^{125}I] gp on the TSK 3000 column, binding activity of the glycoprotein was checked by radioimmunoprecipitation. The fractions were also assayed for radioactivity.

Preparation of the BLV glycoprotein

BLV was grown in fetal lamb kidney cells persistently infected with bovine leukemia virus [7]. The culture fluids were centrifuged at 50 000 g for 90 min. After centrifugation, the pellet was resuspended in 1 ml of 0.05 M disodium hydrogenphosphate (pH 7.0) containing 0.15 M sodium chloride (phosphate-buffered saline, PBS) and loaded onto a gel-permeation column, 90 cm × 1.6 cm (A-2, LKB Productor, Bromma, Sweden). After elution with PBS, the absorbance of the fractions was read at 260 nm. Fractions with readings greater than 0.3 absorbance units were pooled and centrifuged for 2 h at 100 000 g. The virus pellet was then disrupted with 1% Triton X100, and the major surface glycoprotein was purified and iodinated according to the method of Bex et al. [5]. Soluble glycoprotein [8] produced in the culture fluids from the persistently infected lamb kidney cells was also purified using the same method as for gp51 from the virus. Briefly, the culture fluids were precipitated with 50% saturated ammonium sulfate and dialyzed against 0.05 M phosphate buffer. The sample was then placed on a DEAE cellulose column, and the glycoprotein was eluted using a 0–0.5 M sodium chloride gradient. The glycoprotein was then placed on a Lentil lectin affinity column. Before HPGPC, each sample was centrifuged at 10 000 g for 1 min. Approximately 0.1 μCi (3.7 Bq) iodinated viral glycoprotein ([^{125}I]gp51) was injected per analysis. Binding activity of the [^{125}I]gp was tested using a radioimmunoprecipitation assay [5]. Unlabelled BLV glycoprotein was measured using a competition assay similar to the assay for the major internal protein of BLV [9]. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [10] of the [^{125}I]gp was performed using a 5% acrylamide gel. Calibration of the gel was done with ^{14}C-methylated proteins (Amersham, Arlington Heights, IL, U.S.A.). After electrophoresis, the portion of the gel containing the markers was cut off, fixed and impregnated with the scintillation fluor, En^{3}Hance (New England Nuclear, Boston, MA, U.S.A.), and exposed to X-ray film for 24 h at −70°C. Autoradiography was carried out on the remaining portion of the gel after it was wrapped in plastic wrap and frozen at −70°C. Exposure was for 5 h at 70°C with one intensifying screen (Lightning Plus, Dupont, Wilmington, DE, U.S.A.) using Kodak X-Omat AR-5 film.

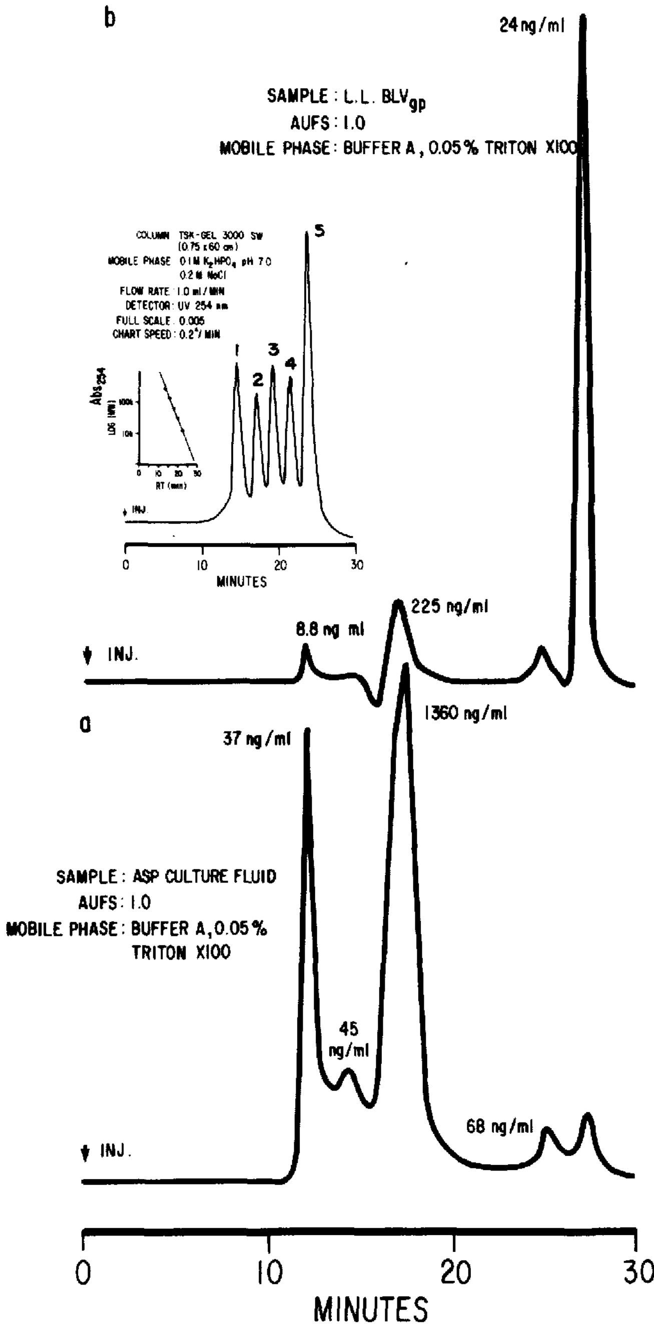

Fig. 1. (a) Chromatogram of ammonium sulfate-precipitated BLV-infected culture fluids. Amount of glycoprotein in each peak was determined by competitive radioimmunoprecipitation assay. (b) Chromatogram of Lentil lectin affinity purified BLV gp. Inset: chromatogram of the markers. The proteins were (shortest to the longest retention times): glutamate dehydrogenase (290 000, peak 1); lactate dehydrogenase (140 000, peak 2); enolase (67 000, peak 3); adenylate kinase (32 000, peak 4) and cytochrome C (12 400, peak 5). Log of the relative molecular mass of the markers versus retention time.

RESULTS

The separation range and calibration of the TSK 3000 column is shown in the inset of Fig. 1. The actual separation range was from 300 000 to 10 000 relative mass units. The column had an inclusion volume of 26 ml and exclusion volume of 12 ml. The chromatogram of culture fluids that had been precipitated with 50% saturated ammonium sulfate is shown in Fig. 1a. The amount of glycoprotein (as determined by competition radioimmunoprecipitation assay) loaded into the column was 5.0 μg, and 80% of this material was found in the peak eluting at a relative molecular mass of 80 000. After the soluble glycoprotein was purified on a Lentil lectin affinity column and a chromatographic profile obtained, 75% of the glycoprotein applied was found in the major glycoprotein peak (Fig. 1b). Another peak outside the inclusion volume was found to be due to the α-D-methylmannoside present in the sample. This peak was not observed if the sample was dialyzed against the mobile phase buffer before chromatography. When purified glycoprotein from a viral preparation was used, the profile was similar to that of the soluble glycoprotein (data not shown).

The chromatographic profile changed when viral glycoprotein was iodinated. Four apparent relative molecular masses were obtained: 100 000, 60 000, 40 000 and 28 000, depending on the additives in the mobile phase. Although only one major band at 68 000 and a minor component at 46 000 were observed on SDS-PAGE for the [^{125}I]gp (Fig. 2d), the chromatographic profile of [^{125}I]gp, when buffer A was the mobile phase, indicated that the protein was heterogeneous with respect to relative molecular mass (Fig. 2a). Two peaks of radioactivity at relative molecular masses of 100 000 and 60 000 and two peaks with absorbance at 254 nm, one in the void column and one at 40 000, were observed. The molecular mass of the [^{125}I]gp with antibody binding activity was a broad peak at 28 000. The major change caused by the addition of 0.05% Triton X100 was the increase of the retention of the major peak to 68 000. A negative peak was introduced that was due to protein binding the Triton X100 from the mobile phase (Fig. 2b). The peak of binding activity was also at a molecular mass of 28 000. With the addition of both α-D-methylmannoside and Triton X100 to the mobile phase, the radioactive peak and the peak of binding activity coincided at a relative molecular mass of 28 000 (Fig. 2c). The analysis of [^{125}I]gp that had been stored for long periods of time showed increased retention times which continued to increase with storage time until they were outside the inclusion volume of the column.

DISCUSSION

The use of HPGPC was an efficient means for separating the soluble and the viral glycoprotein from culture fluids of fetal lamb kidney cells persistently infected with BLV. Recoveries were usually greater than 75% of the total activity applied to the column. This method of purification could then be used to purify material for purposes that require retention of the native structure of the glycoprotein. Iodination of the glycoprotein caused peak broadening and multiple peaks. These could be due to changes induced by radiation damage, structural and chem-

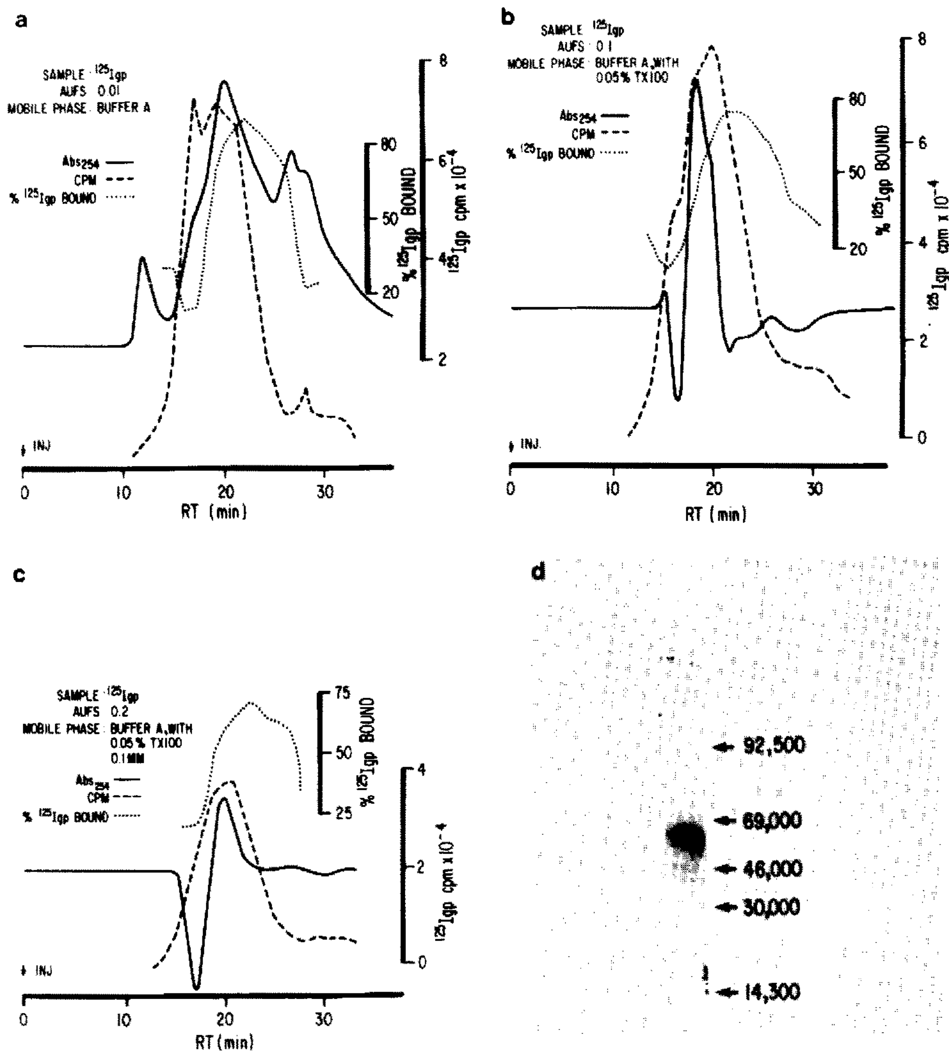

Fig. 2. (a) Chromatogram of [^{125}I]BLV gp with phosphate buffer and sodium chloride as the mobile phase (buffer A). (b) Chromatogram of [^{125}I]BLV gp with 0.05% Triton X100 added to the buffer A. (c) Chromatogram of [^{125}I]BLV gp with 0.05% Triton X100 and 0.1 α-D-methylmannoside added to buffer A. (d) SDS-PAGE of the [^{125}I]gp from BLV. Markers were run on the same gel, the lane cut off, impregnated with the scintillation fluor, En^{3}Hance, and exposed for 24 h at −70°C.

ical alterations from the iodination procedure and possible changes in the amount of glycosylation of the glycoprotein. Another possibility may be due to the interactions of [^{125}I]gp with column matrix. These column interactions could have caused the broad peak observed for the binding activity. The addition of 0.05% Triton X100 to buffer A reduced some of the complexity of the chromatogram, but did not resolve the discrepancies occurring in the molecular masses. The best resolution of the [^{125}I]gp was achieved by adding 0.1 M α-D-methylmannoside and Triton X100 to buffer A. This suggests that [^{125}I]gp does exhibit column

interactions that are reduced by the use of these additives. Possibly other types of commercially available gel-permeation columns may not exhibit these types of column interactions.

We have shown here that HPGPC could be used to purify a viral glycoprotein rapidly and with good recovery. We also found that iodination of the glycoprotein induced changes in the glycoprotein that could be observed using HPGPC. Some of the ambiguities that were found in the profile of the [^{125}I]gp, when compared to SDS-PAGE, were resolved with the addition of a non-ionic detergent and a glucoside to the mobile phase.

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