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Rapid purification of squirrel monkey retrovirus-H major gag protein by high performance liquid chromatography.

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Abstract

The major gag protein (p34) of squirrel monkey retrovirus-H was purified in one chromatographic step by anion-exchange high performance liquid chromatography. The virus in a crude fraction was disrupted with Brij 35 in the presence of three kinds of protease inhibitors. The soluble virus lysate was injected into a Polyanion SI column, and p34 was eluted with a linear salt gradient. The recovery of the protein was about 60%. The purified p34 was nearly homogenous as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining.

KEYWORDS: retrovirus, gag protein, protein purification, high performance liquid chromatography

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—Brief Note—

Rapid Purification of Squirrel Monkey Retrovirus-H Major *gag* Protein by High Performance Liquid Chromatography

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The major *gag* protein (p34) of squirrel monkey retrovirus-H was purified in one chromatographic step by anion-exchange high performance liquid chromatography. The virus in a crude fraction was disrupted with Brij 35 in the presence of three kinds of protease inhibitors. The soluble virus lysate was injected into a Polyanion SI column, and p34 was eluted with a linear salt gradient. The recovery of the protein was about 60%. The purified p34 was nearly homogenous as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining.

Key words : **retrovirus, *gag* protein, protein purification, high performance liquid chromatography**

In the course of our electron microscopic observations of various human lymphoid cell lines, we found a retrovirus produced in a human lymphoblastoid cell line (HLB) (1). Molecular cloning and nucleotide sequencing of the provirus genome integrated in HLB cells revealed that this virus was closely related to squirrel monkey retrovirus (SMRV), and we designated this virus $SMRV_{HLB}$, abbreviated as SMRV-H (2). SMRV-H has a reverse transcriptase with magnesium ion preference and a major *gag* protein of 34,000 daltons (p34) (1). In this study the rapid and simple purification of p34 by high performance liquid chromatography (HPLC) is described.

HLB cells were cultured as previously described (1, 3). A crude SMRV-H fraction was prepared by the treatment of HLB cells with trypsin (1). SMRV-H particles were pelleted by centrifugation at 100,000 $\times g$ for 60 min. The pellet was suspended in a solution (lysis buffer) containing 50 mM Tris-HCl (pH 8.0), 1% Brij 35 (Katayama Chemical Ind., Ltd., Osaka, Japan), 0.8 M NaCl, 2 mM phenylmethansulfonyl fluoride (PMSF, Sigma Chemical Company, St Louis, MO, USA), 15 μg/ml leupeptin, and 15 μg/ml chymostatin. After incubation for 20 min at 0℃ the mixture was diluted two-fold with TBP buffer (50 mM Tris-HCl, pH 8.0, 0.1% Brij 35, and 0.5 mM PMSF). The mixture was centrifuged at 100,000 $\times g$ for 60 min, and the supernatant fluid was pooled as virus lysate. The chromatographic purification of p34 was performed with the Pharmacia FPLC system employing a Polyanion SI HR 5/5 column (Pharmacia Biotechnology, Uppsala, Swe-

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den). The virus lysate was injected into the column which was equilibrated with TBP buffer containing 0.4 M NaCl. The column was washed thoroughly with the equilibration buffer, and proteins retained in the column were eluted with a linear gradient from 0.4 M to 1.2 M NaCl in TBP buffer in 30 min at a flow-rate of 0.5 ml/min. Absorbance was monitored at 280 nm. Fractions (1 ml) were collected, and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (4) and silver staining (5).

The crude SMRV-H fraction was used as the source material for the purification of the major *gag* protein p34. The virion was disrupted with Brij 35, which is a non-ionic detergent with low absorption in the ultraviolet region. Preliminary experiments showed that, when the virus was disrupted by detergent, p34 was immediately degraded by proteases contaminating the crude virus fraction (6). To prevent the degradation of p34, protease inhibitors (PMSF, leupeptin, and chymostatin) were added to the virion lysis buffer, and PMSF was added to all HPLC elution buffers. Protein bands of the crude SMRV-H fraction and virus lysate be-

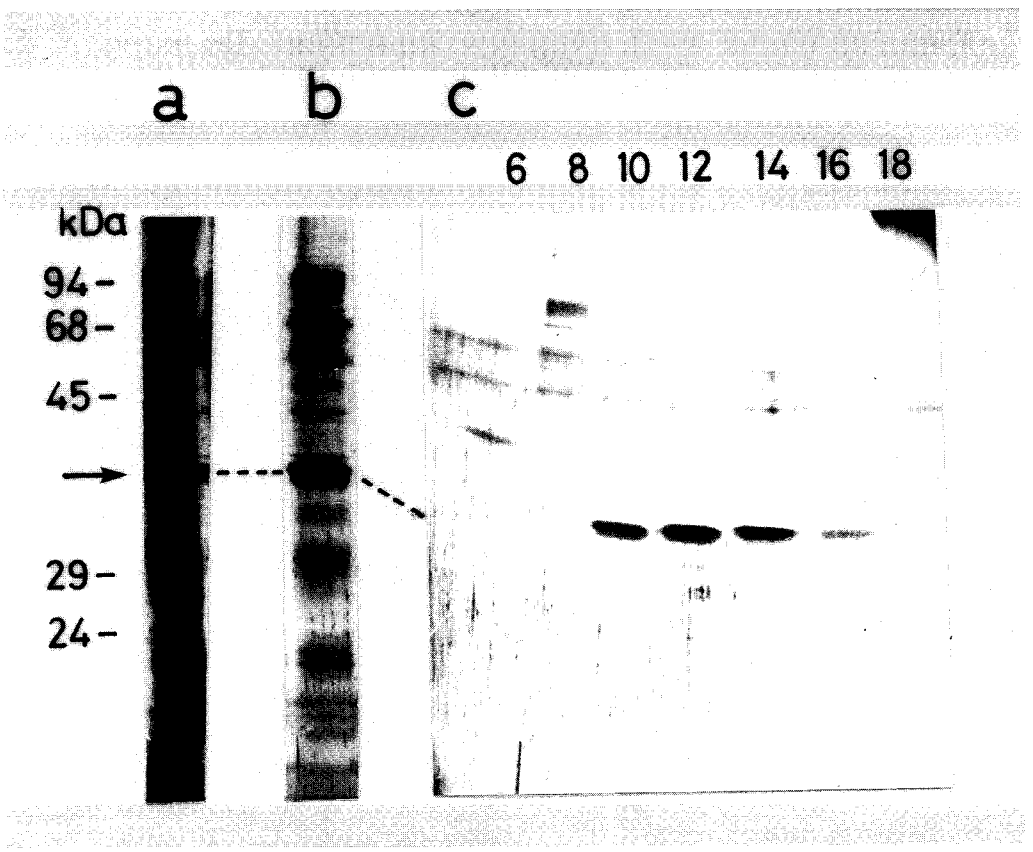

Fig. 1 Purification of SMRV-H major *gag* protein p34 by anion-exchange HPLC. Crude SMRV-H was harvested from HLB cells in one liter of culture medium. The virion was disrupted with 1.5 ml of lysis buffer, and p34 was purified by HPLC as described in the text. Thirty microliter samples were loaded on 12.5% polyacrylamide gel. (a) Crude SMRV-H diluted 4.5-fold, (b) Virus lysate diluted 4.5-fold, (c) HPLC fractions. Numbers of lanes in (c) correspond to the HPLC fraction numbers. The arrow indicates p34.

fore HPLC are shown in Fig. 1. Brij 35 treatment of SMRV-H virions in the presence of protease inhibitors resulted in the efficient extraction of p34 protein without degradation.

The virus lysate was subjected to anion-exchange HPLC. Impurities mainly passed through the column. Protein bands of the fractions eluted with a linear salt gradient are shown in Fig. 1. p34 was recovered at 0.8 M to 1.1 M NaCl in a nearly homogenous form. Broad elution patterns might be the result of polymerization of p34, which tends to aggregate, as characteristic of the core proteins of retroviruses. Recovery of p34 from the column was about 60% as judged by densitometry of stained SDS-PAGE gel. About 25 μg of purified p34 was obtained from HLB cells in one liter of culture medium. The lentivirus *gag* proteins have been purified by reverse phase HPLC with the recoveries of the proteins similar to that of ours (7).

HPLC-purified SMRV-H p34 was useful for biochemical and immunological experiments. The purified p34 had an isoelectric point of 7.3. It was an excellent immunogen for obtaining specific polyclonal antibody against p34 in rats (data not shown). The HPLC method presented in this study was shown to be a rapid method for the purification of the SMRV-H major *gag* protein and might be applied to the purification of the *gag* proteins of other retroviruses.

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References

1. Oda T, Hatsushika M, Watanabe S, Ikeda S, Sumii H, Arakaki Y, Nakamura T, Tsutsui K, Seki S, Akiyama K, Wada T, Nakashima A, Suma F and Murakami M: Immunoelectron microscopic and immunoblotting analyses of a retrovirus produced in a human lymphoblastoid cell line with a monoclonal antibody. Cell Mol Biol (1986) **32**, 343-350.
2. Oda T, Ikeda S, Watanabe S, Hatsushika M, Akiyama K and Mitsunobu M: Molecular cloning, complete nucleotide sequence, and gene structure of the provirus genome of a retrovirus produced in a human lymphoblastoid cell line. Virology (1988) **167**, 468-476.
3. Oda T, Watanabe S, Maki Y, Ikeda S, Seki S, Murakami M, Endo S, Asonuma H and Matsubara T: Authentication of a human lymphoblastoid cell line producing a retrovirus and the infectivity of human T-cell and B-cell lines with the virus. Cell Mol Biol (1988) **34**, 529-537.
4. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (1970) **227**, 680-685.
5. Morrissey JH: Silver stain for proteins in polyacrylamide gels : A modified procedure with enhanced uniform sensitivity. Anal Biochem (1981) **117**, 307-310.
6. Hatsushika M: Analysis of the major *gag* protein of a retrovirus produced in a human lymphoblastoid cell line: Preparation of monoclonal antibodies and amino acid sequence analysis of the N-terminal region. Okayama Igakkai Zasshi (1987) **99**, 1117-1129 (in Japanese).
7. Ball JM, Rao VS, Robey WG, Issel CJ and Montelaro RC: Lentivirus antigen purification and characterization: isolation of equine infectious anemia virus *gag* and *env* proteins in one step by reverse phase HPLC and application to human immunodeficiency virus glycoproteins. J Virol Methods (1988) **19**, 265-277.

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