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Short Communication

Processing of envelope polypeptides of *Herpes simplex* virus type 1 Demonstration of variation in different cell lines by high-performance liquid chromatography and radioimmunoprecipitation

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Abstract

[^{35}S]Methionine-labelled envelope polypeptides of *Herpes simplex* virus type 1, strain F, propagated in mammalian cell culture of various origins, were separated by ion-exchange high-performance liquid chromatography on a TSK DEAE-3SW column. Analysis of the fractions by radioimmunoprecipitation followed by sodium dodecyl sulphate polyacrylamide gel electrophoresis of the immunoprecipitates showed similarities as well as distinct differences in the number, migration patterns and molecular mass of the synthesized polypeptides, depending on the host cell. The results show that this method can be used to demonstrate species-specific or organ-specific differences in the processing of virus-specified polypeptides synthesized in host cells.

1. Introduction

Particles of *Herpes simplex* virus type 1 (HSV-1) contain many virus-encoded polypeptides. Among these are envelope glycoproteins, which serve important biological functions such as cell penetration, virion infectivity and as elicitors of host immune response [1–4]. In the course of HSV-1 replication in infected cells, viral DNA synthesis, protein synthesis, and post-translational processing of nascent polypeptides are greatly influenced by the particular cell type in which the virus is propagated. These differences in viral

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macromolecule synthesis results in genotypic [5–9] as well as phenotypic [10–13] variation among isolates or strains of HSV-1.

Using Con-A affinity chromatography, we recently described variation in the processing of HSV-1 glycoproteins in cell lines from different species [14]. Moreover, we have used high-performance liquid chromatographic (HPLC) methods to separate HSV-1 glycoprotein D [15] and to demonstrate variability in the envelope polypeptide profile of HSV-1 strains [16,17]. In this paper, we report the use of anion-exchange HPLC to demonstrate variation in the envelope polypeptide profile of HSV-1, strain F, propagated in different cell lines.

2. Experimental

2.1. Cells and virus

African green monkey kidney (Vero) and human epidermoid carcinoma (Hep-2) cells and HSV-1 (strain F) were purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). Normal human fibroblast (NHF) cells were obtained from Medical Research Council (MRC, Manchester, UK). Baby hamster kidney (BHK-21/C13) cells were purchased from Flow Laboratories (Irvine, UK). The cells were routinely maintained in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal calf serum (FCS). Penicillin (50 U/ml) and 50 μg/ml streptomycin (PS) were added during all experimental procedures.

2.2. Virus labelling with [^{35}S]methionine

Confluent monolayers of Vero, BHK-21, NHF and Hep-2 cells in 75-cm^2 flasks were infected with the F strain at a multiplicity of infection of 5 plaque forming units per cell. At 4 h post infection, 15 ml of a 10 μCi/ml solution of [^{35}S]methionine (1000 Ci/mmol, Amersham, Buckinghamshire, UK) in methionine-free EMEM supplemented with 2% FCS and PS was added to each flask. After 48 h, the medium was replaced with EMEM containing 2% FCS, PS and 2 m*M* L-methionine and incubated for 2 h. The infected cells were washed three times with phosphate buffered saline (PBS), and virus particles were purified as previously described [18].

2.3. Extraction of [^{35}S]-labelled envelope polypeptides

Purified virus from various cells was resuspended in 0.5 ml of 50 m*M* Tris-HCl, pH 7.5, 1% reduced Triton X-100, 1 m*M* phenylmethylsulphonyl fluoride and incubated on ice for 1 h. The cell extract was centrifuged at 48 000 *g* in a Beckman TL 100.3 rotor for 30 min at 4°C. The supernatant was recovered and stored at − 70°C.

2.4. HPLC hardware

The HPLC system was from LKB (Bromma, Sweden). For the anion-exchange procedure, it consisted of two Model 2150 titanium head pumps, a Rheodyne Model 7125 injector (Cotati, CA, USA), a Model 2151 variable wavelength monitor, a Model 2152 controller and a Model 2212 Helirac fraction collector. A Supelco TSK DEAE-3SW anion-exchange column (Bellefonte, PA, USA; 75 × 7.5 mm I.D., 10 μm particle size) was used.

2.5. Anion-exchange HPLC

Following the injection of 100 μl (50 μg protein) of detergent extract, the column was washed for 20 min with 50 m*M* Tris-HCl, pH 7.5, containing 0.1% reduced Triton X-100 (Buffer A). Adsorbed polypeptides were eluted by a 30-min linear gradient of 0–0.5 *M* NaCl in Buffer A (Buffer B). The flow-rate was 0.5 ml/min and 1-min fractions were collected. Absorbance was monitored at 280 nm. Fractions from three consecutive runs were pooled and aliquots (25 μl) were taken from each fraction, mixed with 5 ml of Optifluor scintillation liquid (Packard, Downer's Grove, IL, USA) and counted in LKB Rackbeta scintillation counter.

2.6. Radioimmunoprecipitation

Lyophilized HPLC fractions were resuspended

in 0.5 ml of sterile water followed by the addition of 10 μl of 10% bovine serum albumin (BSA, Sigma, St. Louis, MO, USA; Cat. No. 2518). Five μl of affinity purified, rabbit anti-HSV-1 IgG (Accurate Chemical, Westbury, NY, USA; Cat. No. AXL 237) were added and the mixture was incubated overnight at 4°C. Immune complexes were precipitated by the addition of 200 μl of a 10% suspension of protein A–Sepharose (Pharmacia/LKB, Uppsala, Sweden) for 1 h at 4°C. The beads were washed twice with 0.5 ml of 10 m*M* Tris-HCl, pH 7.2, containing 0.5 *M* NaCl and 0.1% reduced Triton X-100, and once with 0.5 ml of 10 m*M* Tris-HCl, pH 7.2.

2.7. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Precipitated immune complexes were resuspended in 50 μl of sample buffer (62.5 m*M* Tris-HCl, pH 6.8, containing 10% glycerol, 2% SDS, 5% ß-mercaptoethanol, and 0.02% bromophenol blue), boiled for 3 min, and separated by electrophoresis in 12% polyacrylamide gels as described by Laemmli [19]. ^{14}C-Labelled protein molecular weight markers (Amersham) were used: myosin, phosphorylase b, BSA, ovalbumin, carbonic anhydrase and lysozyme, with molecular mass (M_r) of 200, 92, 69, 46, 30, and 14 kDa, respectively. Gels were fixed in 20% methanol/10% acetic acid overnight, rinsed with water and then soaked in 1 *M* sodium salicylate, 2% glycerol (v/v) for 2 h. They were dried under vacuum and exposed to a Kodak X-ray film (XK-6) with intensifying screens at −70°C.

3. Results and discussion

HPLC is a fast, reliable and versatile analytical tool which has increasingly been employed for the separation and purification of proteins [20,21]. It is especially well-suited for the analysis of viruses since it requires only a minimal amount of sample. However, only a few studies have reported the use of HPLC to separate viral membrane proteins [15,22–25]. In this report, we describe the separation of detergent solubilized polypeptides of HSV-1 (strain F) propagated in different cell lines, using anion-exchange HPLC under non-denaturing conditions. Reduced Triton X-100 was selected for membrane solubilization because of its low UV absorbance [26] and its mild detergent properties which maintain the extracted polypeptides in their native state.

The elution profile of the extracted radiolabelled envelope polypeptides of HSV-1 propagated in Vero cells (Fig. 1a) and Hep-2 cells (Fig. 2a) showed four similar peaks, designated as A, B, C and D in the order of elution. In NHF cells, there were three peaks A, B and C (Fig. 3a), whereas BHK-21 cells showed two peaks A and B (Fig. 4a). All peaks were eluted with buffer B except those designated A.

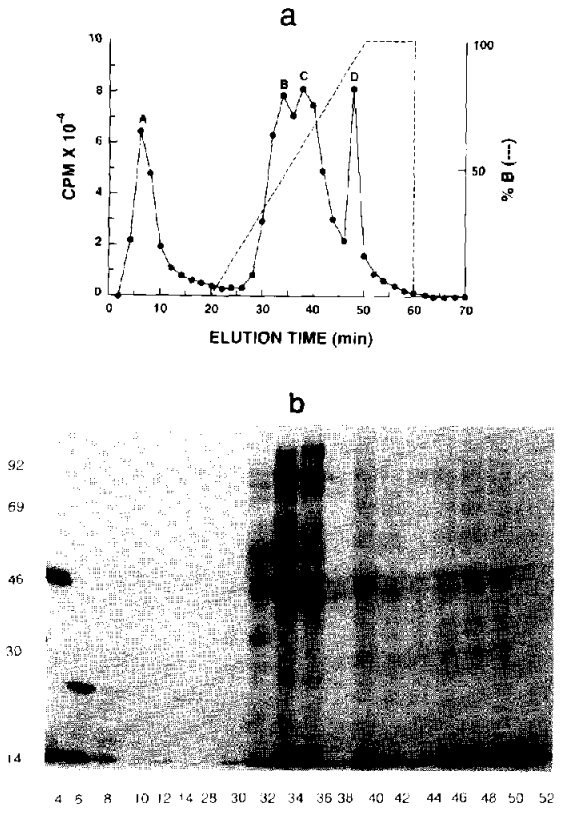

Fig. 1. Profile of HSV-1 envelope polypeptides extracted from Vero cells by anion-exchange HPLC on a TSK DEAE-3SW column (a) followed by radioimmunoprecipitation and SDS-PAGE of HPLC fractions (b). Buffer composition and molecular mass markers are described in the text.

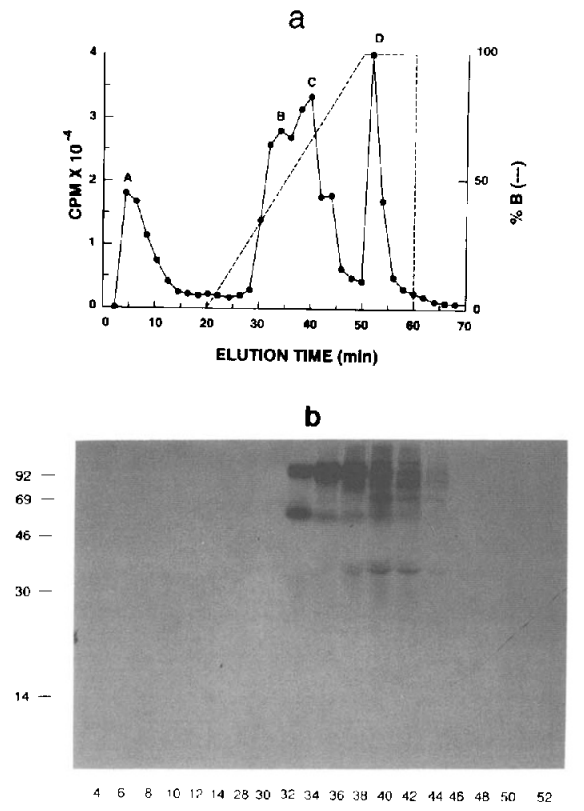

Fig. 2. Profile of HSV-1 envelope polypeptides extracted from Hep-2 cells by anion-exchange HPLC on a TSK DEAE-3SW column (a) followed by radioimmunoprecipitation and SDS-PAGE of HPLC fractions (b). Buffer composition and molecular mass markers are described in the text.

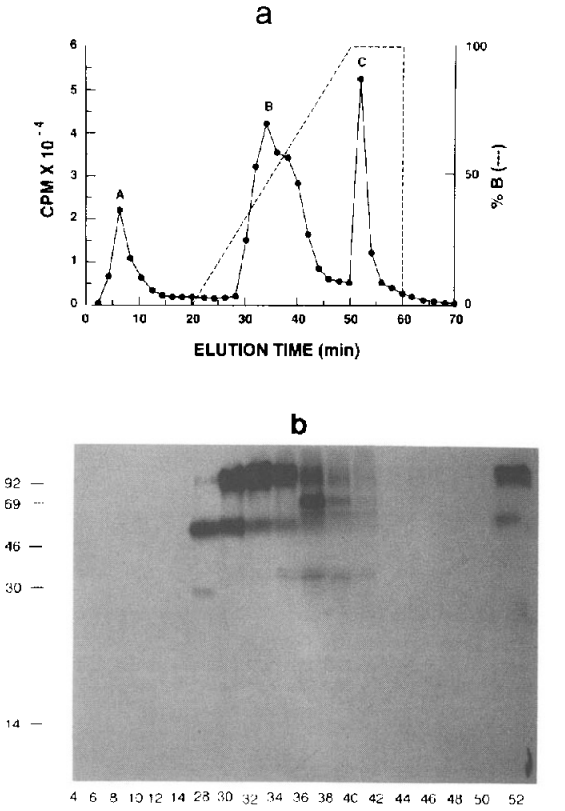

Fig. 3. Profile of HSV-1 envelope polypeptides extracted from NHF cells by anion-exchange HPLC on a TSK DEAE-3SW column (a) followed by radioimmunoprecipitation and SDS-PAGE of HPLC fractions (b). Buffer composition and molecular mass markers are described in the text.

The composition of the peaks was examined to determine the variances in the polypeptides synthesized in the different host cells. Therefore, HPLC fractions were immunoprecipitated and subjected to SDS-PAGE analysis. The resulting autoradiograms are shown in Figs. 1b–4b. Peak A representing unabsorbed material in each cell line (fractions 4–12), consisted of low M_r polypeptides of 45 and 22 kDa in Vero cells (Fig. 1b). Peak A of Hep-2 (Fig. 2b) and NHF (Fig. 3b) cells did not show any polypeptide, whereas the BHK-21 cells (Fig. 4b) contained three predominant polypeptides of apparent M_r of 85, 32 and 14 kDa. Peak B (fractions 28–36 for all cells except for BHK-21 where it was fractions 28–46) was eluted in a narrow low salt concentration range of 0.2–0.25 *M* NaCl in each cell line. This peak was highly enriched in high-molecular-mass polypeptides (M_r 56–125 kDa), which are probably acidic glycoproteins. Four polypeptides with M_r of 115, 110, 85 and 56 kDa were common to all cells. Two polypeptides with low M_r of 45 and 43 kDa were present in both Vero and BHK-21 cells, but absent in the other two human cells. Vero cells showed two unique polypeptides with M_r of 90 and 72 kDa which were not present in the other three cells. A 32 kDa polypeptide was faintly present in Vero, BHK-21 and NHF cells, but totally absent in Hep-2 cells. Hep-2 cells showed two unique polypeptides with M_r of 125 and 68 kDa. NHF and BHK-21 cells did not show any unique polypeptide.

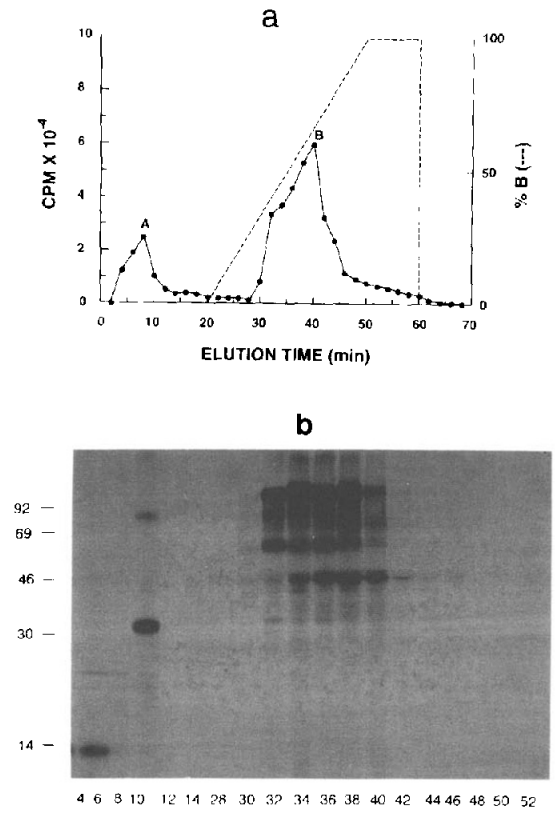

Fig. 4. Profile of HSV-1 envelope polypeptides extracted from BHK-21 cells by anion-exchange HPLC on a TSK DEAE-3SW column (a) followed by radioimmunoprecipitation and SDS-PAGE of HPLC fractions (b). Buffer composition and molecular mass markers are described in the text.

Peaks C and D (fractions 38–52) were eluted in a salt concentration of 0.3 *M* and 0.5 *M* respectively in Vero, Hep-2 and NHF cell lines. Variable polypeptides were also found in these fractions depending on the cell lines. Vero cell line contained residual amounts of the 45 and 43 kDa low M_r polypeptides whereas in Hep-2 cell line very few polypeptides were present. In contrast, peak D of NHF contained the three polypeptides of M_r of 115, 110, and 56 kDa described previously in peak B.

Differential processing of nascent HSV polypeptides in various cells and among different strains has been described. Soslau *et al.* [13] described the differences in HSV proteins produced in blood-derived, Vero and Hep-2 cells, particularly gB and gD by monoclonal antibodies. Al-Ahdal *et al.* [14] have shown that cells from various species process HSV-1 glycoproteins differently. In a recent study by Al-Ahdal [27], glycoprotein variability of various strains of HSV-1 propagated in the same cell line was demonstrated by monoclonal antibodies. In this study we employed anion-exchange HPLC followed by immunoprecipiation to demonstrate the differences in the electrophoretic pattern of polypeptides identified in the fractions from each cell line, indicating the dependence on the host cell for post-translational protein modification during HSV-1 replication. It is important to demonstrate such variation since HSV-1 can multiply in different kinds of human cells *in vivo*. The resultant strains may differ from one another in terms of antigenic structure and concentration, which may influence the mode of detection and treatment. Furthermore, the antigenic variation will have a strong impact on vaccine development.

4. References

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