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COMPARATIVE POLYPEPTIDE ANALYSIS OF HUMAN, MURINE AND STRIGIS HERPESVIRUSES WITH MURINE CYTOMEGALOVIRUS BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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Summary. — The polypeptide composition of five purified murine herpesvirus (MHV) strains grown in a stable line of rabbit embryo fibroblasts (REF) was analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and compared with herpes simplex virus type 1 (HSV-1). About 24 structural polypeptides of molecular mass ranging from 275 000 to 25 000 were identified in MHV and HSV-1. The polypeptide profiles of MHV and HSV-1, showed a close similarity. The polypeptides of MHV were further compared with those of HSV-1, HSV-2, herpes virus strigis (HVS) and murine cytomegalovirus (MCMV). Differences were found between herpesviruses of different origin and MCMV. SDS-PAGE analysis of the six strains of MCMV labelled with ^{14}C-amino acid hydrolysate also revealed differences in electrophor eticprofiles of MHV and MCMV proteins, what was confirmed by densitometric scanning of HSV-1, MHV and MCMV.

Key words: murine herpesviruses; human herpesviruses 1 and 2; herpesvirus strigis; murine cytomegalovirus; virus purification; polyacrylamide gel electrophoresis; polypeptides; autoradiography; densitometry

Introduction

Biological properties of herpesviruses isolated from different organs of free-living small rodents and studies on their experimental pathogenesis indicated, that these viruses would belong to subfamily *Alphaherpesvirinae* (Blaškovič *et al.*, 1980; Čiampor *et al.*, 1981; Svobodová *et al.*, 1982a, b; Blaškovič *et al.*, 1984; Mistríková and Blaškovič, 1985; Rajčáni *et al.*, 1985). Nevertheless, some biological properties of the murine herpesviruses (MHV) resembled to murine cytomegalovirus (subfamily *Betaherpesvirinae*). Further data concerning classification of MHV were coming from comparison in

sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of the polypeptides of purified MHV, HSV-1, HSV-2, HVS and MCMV virions. These results complemented with autoradiography and densitometric tracing of the herpesvirus polypeptides are the subject of this presentation.

Materials and Methods

Cells and media. Stable line of rabbit embryo fibroblast cells (REF, kindly provided by Dr. D. Řezáčová, Institute of Sera and Vaccines, Prague), African green monkey kidney cells (VERO), chick embryo fibroblast cells (CEC) and primary mouse embryo fibroblast cells (MEC) were all cultured in Eagle's basal medium (BEM) supplemented with 5—10% inactivated bovine serum (IBS), glutamine (3 g per 100 ml) and antibiotics (100 units of penicillin and 100 μg of streptomycine per ml). These cells were used throughout all experiments as well as for virus stock production. Mouse embryo fibroblasts were prepared by trypsinization of 12-to 16-day-old embryos obtained from outbred white mice (Dobrá Voda breed). The cells were propagated in BEM, supplemented with 10% IBS, glutamine and antibiotics (as given above).

Viruses. Six strains of MHV isolated from *Clethrionomys glareolus* (No. 60, 68, 72) and *Apodemus flavicollis* (No. 76, 78 and Šumava A. f.) (Blaškovič *et al.*, 1980; Mistríková and Blaškovič, 1985) were used. These strains were routinelly propagated in REF cells. HSV-1, strain HSZP (Szántó *et al.*, 1972), was propagated also in VERO cells, HSV-2 strain only in REF cells. Herpesvirus isolated from an owl (herpesvirus strigis — HVS) was obtained from Prof. F. Bürki (Faculty of Veterinary Medicine, University of Vienna, Austria). It was propagated in CEC cultures. Murine cytomegalovirus (MCMV), the Smith strain, was obtained from the American Type Culture Collection, Rockville, Md., U.S.A., by courtesy of WHO. It was propagated in MEC cultures.

Purification of viruses. Purified viral preparations were obtained by differential centrifugations, discontinuous Ficoll and sucrose gradient centrifugation as described previously (Svobodová *et al.*, 1982*b*). Protein content of purified preparations was measured according to Lowry *et al.* (1951) with bovine serum albumin as standard. In the case of MCMV, the extracellular virus was used as starting material for purification procedures (Fiala *et al.*, 1976).

Infection and labelling of the cells. Confluent cell monolayers (approximately 2×10^6 cells per flask) were infected at input multiplicity (MOI) of 2—4 $TCID_{50}$ per cell and kept for 60 min at 37 °C. MOI of 5—10 per cell was used in the case of MCMV. After virus adsorption, the inoculum was removed and replaced with BEM supplemented with 5% IBS. At 24—48 hr post-infection (p.i.), when first cytopathic changes occurred, 2 ml BEM/10 (ten times reduced amino acids except of arginine and supplemented with 1% IBS) containing 0.1 MBq/ml of ^{14}C-amino acid hydrolysate (ÚVVVR, Prague, specific activity 4×10^4 MBq/g) was added. At the end of the labelling period (48—96 hr p.i.) the cells were rinsed with ice-cold phosphate-buffered saline (3×5.0 ml/flask) scraped off, pelleted and stored frozen at −80 °C until subsequent polyacrylamide gel electrophoresis. To prepare uninfected controls, the cells were labelled for a period similar as described above.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE), autoradiography and densitometry. Samples were analysed on 8% polyacrylamide gel (cross-linked with methylenbisacrylamide) as described by Matis and Rajčáni (1980). The gels were fixed and treated with 1 mol/l sodium salicylate to provide fluorographic enhancement (using Medix-Rapid X-ray film, Hradec Králové). The following proteins were used for molecular mass determination: myosin, beta-galactosidase, phosphorylase B, bovine serum albumin, ovalbumin and carbonic anhydrase (Sigma). Densitometry tracing of the autoradiographs were done using a laser densitometer (LKB).

Results

In preliminary experiment, the electrophoretic mobility of the polypeptides of (5) MHV strains (No. 60, 68, 72, 76 and 78) and of HSV-1 were compared (Fig. 1). Coomassie brilliant blue-stained polyacrylamide gels showed well recognisable bands with both viruses. At the level corresponding to

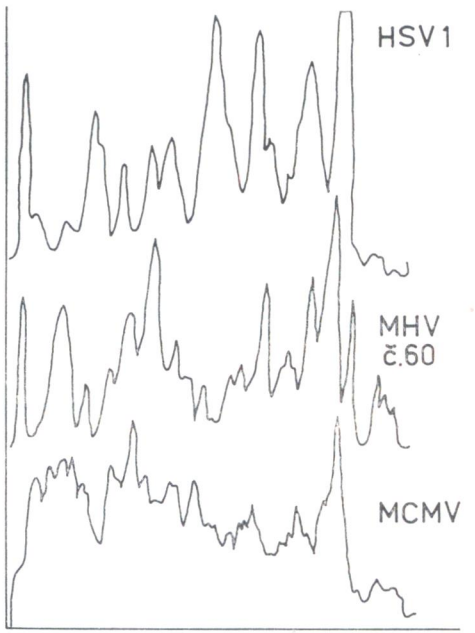

Fig. 4
Densitometric tracing from the autoradiograph HSV-1, MHV No. 60 and MCMV. The ^{14}C-amino acid labelled protein were analysed in 8% acrylamide gel.

the major capsid polypeptide 155 K of HSV-1 (marked with number 3), similar bands of the five MHV were located. Bands corresponding to glycoproteins C, B and A of HSV-1 with a molecular mass in the range of 130 K to 119 K (Fig. 1) were also visible, but showed some variation. Polypeptide bands in the range of HSV glycoproteins E (87 K) and D (59 K to 65 K) could be distinguished as well (Fig. 1).

Polypeptide profiles of purified virions from 4 MHV, HSV-1, HSV-2,HVS and MCMV stained with Coomassie briliant blue in SDS-PAGE are presented in Fig. 2. The major 155 K capsid polypeptide could be found in all 4 MHV and in HSV-1, but was not distinct in HSV-2, MCMV and HVS. The glycosylated proteins of apparent mol. mass from 130 K to 119 K could be traced in all MHV strains, in HSV-1, HSV-2 but less in HVS. The bands of polypeptides 59—65 K and 87 K showed some similarity. The major MCMV polypeptide 140 K was visible. Other bands (below 50 K) of MHV and HSV-1, HSV-2 revealed a great similarity.

Further analysis of virus coded polypeptides was performed using ^{14}C--labelled amino acid hydrolysate added to medium fluid at different time intervals (6, 20 and 48 hr) p.i. depending on cytopathic effect formation. REF and MEC cells served as controls. HSV-1 grown in REF and VERO cells, the original five MHV strains (No. 60, 68, 72, 76, 78) grown in REF cells and the recently isolated MHV, strain Šumava A.f. (Mistríková and Blaškovič, 1985) were used in comparison with MCMV (Fig. 3). The major

herpesvirus polypeptide 155 K of MHV and HSV-1 seemed practically identical. Very well visible was the major 140 K MCMV protein. Also other bands of polypeptides of herpesviruses showed identity or very close resemblance. Some differences could be recognised in the region of polypeptides with molecular weight below 44 K. Striking differences were found in the polypeptide profiles between alphaherpesviruses and MCMV.

Densitometric tracing of the SDS-PAGE of HSV-1, MHV, strain No. 60 and MCMV (Fig. 4) confirmed a certain degree of similarity between apparent molecular mass of the two alphaherpesvirus (human and murine) polypeptides with some quantitative differences. The densitometric profile of MCMV was different, although some polypeptides with identical molecular mass could be recognised in members of both subfamilies.

Discussion

The search for proper determination and classification of five strains of murine herpesviruses (Blaškovič *et al.*, 1980) into the subfamily *Alpha-* and *Betaherpesvirinae* several criteria have been used: morphology of the virion and its assembly in cells studied by electron microscopy (Čiampor *et al.*, 1981), requirements for growth of the virus in cell cultures originating from different animal species (Svobodová *et al.*, 1982a), antigenic relatedness to human and animal alphaherpesviruses and to murine cytomegalovirus (Svobodová *et al.*, 1982*b*), experimental pathogenesis in mice of different age groups (Blaškovič *et al.*, 1984; Rajčáni *et al.*, 1985), ecology of the virus under natural conditions (Mistríková and Blaškovič, 1985). Preliminary conclusion to classify these viruses into subfamily *Alphaherpesvirinae* (Svobodová *et al.*, 1982*b*) was confirmed by the recent isolation from lung tissue of the trapped *Apodemus flavicollis* rodent (Mistríková and Blaškovič, 1985) inoculated directly on REF cell cultures. A further attempt was made to support our preliminary assumption by SDS-PAGE analysis of structural proteins of highly purified alphaherpesviruses and autoradiography of proteins synthesized in REF used to grow the murine herpesvirus, HSV-1 and murine cytomegalovirus.

In this respect, the papers of Kim *et al.* (1976), Killington *et al.* (1977), Fiala *et al.* (1976) and Siqueira-Linhares *et al.* (1981) offered valuable data on the significance of individual polypeptide bands in SDS-PAGE. When comparing the electrophoretic profiles of purified MHV, HSV-1, HSV-2 and HVS, similarity, although not a complete identity of the electrophoretic mobility of their 24 polypeptide bands could be seen. Our results resemble those obtained by Killington *et al.* (1977). It could be concluded, that the mice alphaherpesviruses under study could be considered as members of this subfamily. A decisive role has the position of the major 155 K polypeptide and that of glycoproteins. We consider as characteristic for the MCMV the presence of 140 K major protein. There was a striking difference in the position of polypeptide bands in SDS-PAGE between MHV and MCMV. The confirmation of polypeptide analysis of purified virions in SDS-PAGE was

made by autoradiography and by densitometric profiles of the polypeptides from three selected viruses.

The polypeptide analysis of purified herpesviruses in SDS-PAGE and the autoradiography may serve as a helpful criterion for classification of viruses from the family *Herpesviridae*. Comparison of the members of this large family by means of SDS-PAGE, on the other hand, testifies the similarity or perhaps the identity of certain structural (virion) proteins.

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Explanation to Figures (Plates I—III):

Fig. 1. Electrophoretograph of purified MHV virions no. 60, 68, 72, 76, 78 and HSV-1 in 8 % SDS-PAGE. Viral proteins have been numbered 1—14. Molecular weight marker run simul-

taneously was bovine albumin (68 K). Molecular mass of polypeptide bands: 1 = 275 K, 2 = 184 K, 3 = 155 K, 4 = 126 K, 5 = 98 K, 6 = 87 K, 7 = 71 K, 8 = 65 K, 9 = 59 K, 10 = 50 K, 11 = 43 K, 12 = 37 K, 13 = 33 K, 14 = 25 K.

Fig. 2. Electroforetograph of purified MHV virions no. 60, 72, 76, 78, HSV-1, HSV-2, MCMV and HVS in 8 % SDS-PAGE (m.w. in the right in kDa)

Fig. 3. SDS-polyacrylamide analysis of the ^{14}C-amino acid-labelled polypeptides synthesized in uninfected REF and MEC cells and in REF, VERO and MEC cells infected with HSV-1, MHV and MCMV (m.w. of standard proteins in the right in kDa).