Synthesis and processing of a gp28/32 membrane glycoprotein induced by Marek's disease virus serotype 2

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The post-translational events leading from the precursor to the processed forms of a glycoprotein with an Mr of 28K to 32K (gp28/32) of Marek's disease virus (MDV) serotype 2 were examined with pulse-chase experiments and treatment with tunicamycin and monensin. Cell-free translation of infected cell mRNA followed by immunoprecipitation analysis suggested that a polypeptide with a size of 22K is the initial precursor. Experiments with endo-β-N-acetylgalactosaminidase H and endo-β-N-acetylgalactosaminidase F indicated that gp28/32 contains mostly N-linked oligosaccharides of the complex type. These studies showed that 22K, the initial product, is then processed through intermediates to the 28K to 32K form.

Marek's disease virus (MDV) is the aetiological agent of Marek's disease (MD), a highly contagious malignant lymphoma of chickens. On the basis of immunological analyses, various strains of MDV-related viruses have been subdivided into three serotypes. Attenuated MDV serotype 1 (MDV1), MDV serotype 2 (MDV2) and herpesvirus of turkeys (HVT) are employed as effective vaccines against MD.

The glycoproteins located on the viral envelope and on the surface of the virus-infected cells are involved in the major interactions with the host immune system (Carter et al., 1981; Eberle & Courtney, 1980), and thus are important in conferring protective immunity. Therefore, the analysis of glycoproteins is essential for the construction of improved vaccines. We initiated a series of studies aimed at identifying and characterizing viral proteins that are targets of the immune responses during MDV infections (Kato & Hirai, 1985). We have previously described the characterization of two groups of MDV-related virus-induced glycoproteins, A (gA) and B (gB), recognized by monoclonal antibodies (MAbs) (Kato & Hirai, 1985). The existence of these glycoproteins was shown in MDV1-, MDV2- and HVT-infected cells (Hirai et al., 1986). However, relatively little is known about their specific biological functions.

Recently we reported the isolation of a panel of MAbs against MDV2 (Nakajima et al., 1989). Using these MAbs, seven kinds of MDV2-induced polypeptides were identified. Among them, a glycoprotein with an Mr of 28K to 32K (gp28/32), which is different from the previously identified gA and gB, was detected on the surface of MDV2-infected cells and in the cytoplasm by an immunofluorescence test with MAbs reactive with gp28/32. The MAbs reacted with a 25K/29K polypeptide in MDV1-infected cells, but not with any polypeptides in HVT-infected cells. Here we describe the characterization of a third group of MDV-related virus-induced glycoproteins (gp28/32) recognized by MAbs against MDV2.

The virus strain HPRS24 of MDV2 was grown in chick embryo fibroblasts (CEF) prepared as described previously (Nakajima et al., 1986). Labelling of mock-infected and infected cells with [35S]methionine (1250 Ci/mmol; Amersham) and pulse-chase experiments were done as described previously (Ikuta et al., 1983, 1985). For labelling in the presence of tunicamycin (TM; Sigma) at a concentration of 2 μg/ml or monensin (Calbiochem-Behring) at a concentration of 10⁻⁶ M, cells were preincubated with these drugs for 1 h, and then labelled with [35S]methionine in the presence of these drugs. The techniques used for preparation of MAbs and immunoprecipitation followed by SDS-PAGE (separation gel, 10%; spacer gel, 4%) or by two-dimensional gel electrophoresis were as described previously (Nakajima et al., 1986). Endo-β-N-acetylgalactosaminidase H (endo H) (10 μl of 1 unit/ml solution: Boehringer Mannheim) or endo-β-N-acetylgalactosaminidase F (endo F) (0·25 units; Boehringer Mannheim) were added to samples as described by Sithole et al. (1988). The polyadenylated poly(A)⁺ RNA fraction was isolated on oligo(dT)–cellulose (Collaborative Research) from total RNA extracted by the method of Chirgwin et al. (1979).
Poly(A)+ RNA was translated in vitro using a rabbit reticulocyte lysate (Amersham) according to the instructions of the supplier. Portions of the in vitro translation reaction mixtures were immunoprecipitated and then subjected to SDS–PAGE.

To examine whether the gp28/32 polypeptides are composed of both precursor and processed forms, cells infected with the HPRS24 strain of MDV2 were pulse-labelled and then chased as shown in Fig. 1(a). Cell lysates prepared after each chase period were treated with MAb C47, reactive with gp28/32 of MDV2. The processing steps of gp28/32 are shown in Fig. 1(a). Two polypeptides of 26K and 28K were detected after 10 min pulse labelling in infected cells and then polypeptides with a heterogeneous mobility of 28K/32K were found after a chase of 30 min and 60 min, indicating 26K and 28K were precursor forms of gp28/32 (Fig. 1a). Furthermore, virus-infected cells at 48 h post-infection (p.i.) were labelled for 4 h with [35S]methionine in the presence of TM. TM is known to interfere with the formation of the N-acetylglucosamine pyrophosphoryldolichol intermediate, and thus to inhibit the synthesis of N-linked oligosaccharide side-chains (Heifetz et al., 1979). Inhibition of N-linked glycosylation by TM resulted in identification of the other precursor polypeptide of 22K, indicating that gp28/32 contains N-linked oligosaccharides (Fig. 1b). To identify further the complete nascent polypeptides of gp28/32, a cell-free translation experiment was carried out. Cell-free translation of HPRS24 strain-infected cell mRNA, followed by immunoprecipitation analysis using a MAb against gp28/32, revealed a primary translation product of 22K, suggesting that the 22K polypeptide is the initial precursor (Fig. 1c). Since glycosylation of polypeptides is not expected to occur in the rabbit reticulocyte in vitro translation system (Rothman et al., 1978), the precursor forms detected in TM-treated cells are the complete nascent polypeptides of gp28/32.

We further studied the effect of monensin which is an ionophore and blocks transport of glycoprotein from the Golgi apparatus to the plasma membranes. Virus-infected cells at 48 h p.i. were also labelled for 4 h with...
[35S]methionine in the presence of monensin and then extracts from infected cells were immunoprecipitated with MAb C47. 27K/31K polypeptides were observed in the presence of monensin (Fig. 1d). Therefore, monensin prevented the final processing steps of gp28/32, suggesting that transport through the Golgi apparatus is required for its complete processing. Next, to define the type of oligosaccharide chains on gp28/32, the polypeptides immunoprecipitated with MAb C47 were digested with Endo H or Endo F. Endo H is known to cleave between the two proximal N-acetylglucosamine residues of the high-mannose type oligosaccharides, and Endo F cleaves N-linked oligosaccharides of both the high-mannose and complex types. Fig. 1(e) shows that gp28/32 was cleaved to 24K and 22K polypeptides with Endo F, but not with Endo H, indicating that gp28/32 contains complex-type carbohydrate chains.

For determination of the isoelectric points (pIs) of the gp28/32 polypeptides, HPRS24 strain MDV2-infected cells and mock-infected cells labelled with [35S]methionine were analysed by two-dimensional gel electrophoresis using isoelectric focusing followed by SDS–PAGE (Fig. 2). The patterns of gp28/32 in cells infected with HPRS24 obtained by two-dimensional gel electrophoresis were six spots with pIs of 5.0, 5.3, 5.4, 5.6, 5.7 and 5.9. When we compared these values for gp28/32 with those previously reported for gA and gB (Long et al., 1975; Velicer et al., 1978; Ikuta et al., 1985), the pIs of gA were more acidic and those of gB were more basic. From these results, gp28/32 seems structurally different from previously identified gA and gB.

The results presented here have elucidated the synthesis and processing of gp28/32. MAbs were used to gain information about the biosynthesis of gp28/32. These include the carbohydrate structure and the processing steps at the post-translational level.

A remarkable degree of structural conservation has been shown among proteins of herpesviruses (Snowden et al., 1985). Among MDV-induced glycoproteins, gA was shown to have significant homology to herpes simplex virus (HSV) gC, varicella-zoster virus gP and pseudorabies virus gII (Binns & Ross, 1989; Kato et al., 1989). Furthermore, a homologue of HSV gB was also identified in MDV1-infected cells (Ross et al., 1989). These homologues among various herpesviruses may play a common role in virus growth. The predicted Mr of 25237 for the HSV-1 glycoprotein gG encoded in the US4 region is similar to that of gp28/32 although the size of the processed form is different from gp28/32 (Frame et al., 1986). In addition, the gp28/32 complex is very similar in size to gp22/26K of murine cytomegalovirus (Loh, 1989). However, the structural similarities and cross-reactivities between them are not known. Experiments are in progress to investigate the possible biological functions of gp28/32 and to identify the coding regions for gp28/32 in the MDV genome.

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References


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