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Fatty acid acylation of viral proteins in murine hepatitis virus-infected cells

Brief Report

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Summary

The fatty acid acylation of the cell-associated virus-specific proteins of mouse hepatitis virus (A 59-strain) was studied. ^{3}H-palmitate label was associated with E 2, one of the two virion glycoproteins and its intracellular precursor gp 150. A 110 K protein, the unglycosylated apoprotein of gp 150, accumulated by tunicamycin treatment, also incorporated radiolabeled palmitic acid. The addition of fatty acid to the MHV-A 59 E 2 protein is therefore not dependent on glycosylation.

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Covalent fatty acid binding is a general feature of a large variety of glycoproteins (12). Also many viral glycoproteins are acylated. The role of this fatty acid acylation is unknown; it may be involved in intracellular transport of viral proteins (21) and would thus have an important biological function for virus assembly and budding (14). Indeed does inhibition of fatty acid acylation, using the antibiotic cerulenin, effectively block the formation and release of virus particles (10). Acylation is a post-translational event; the glycoproteins of vesicular stomatitis virus (VSV) and Sindbis virus are modified during the late stages of maturation (14). In Semliki forest virus (SFV) infected cells, however, the precursor polypeptide of the structural SFV glycoproteins serves as the primary acceptor of acyl chains (1). This means that acylation can also take place during the early stages of the maturation of viral proteins. Recently, Berger and Schmidt (2) described that the enzyme fatty acyltransferase, which acylates the precursor protein of SFV, is associated with the rough endoplasmic reticulum.

We examined the fatty acid modification of viral proteins in mouse hepatitis virus-infected cells (strain MHV-A 59, obtained from the American Type Culture Collection, Rockville, MD). MHV-A 59 is a member of the Coronaviridae, a family of lipid-enveloped viruses with a single strand of infectious RNA of about 6×10^6 molecular weight (19, 20). The viral envelope contains two different glycoproteins: a transmembrane protein (E 1) with a mol. wt. of about 26.5 kd and two species of surface proteins (E 2), which carry the epitopes eliciting virus-neutralizing antibodies (9). The two glycosylated protein species of E 2 with mol. wts. of 90 kd are processed by proteolytic cleavage from a 180 K protein. Only one of the 90 kd E 2 species, 90 A, is acylated, suggesting that it is associated with the lipid bilayer (18). In infected cells a 150 K glycosylated protein species (gp 150) is encountered, which is the precursor of the uncleaved 180 kd form of E 2. In the presence of tunicamycin (an inhibitor of N-glycosylation) a nonglycosylated E 2 precursor protein (110 K) is found instead (9). In this report we present evidence that not only the E 2-glycoprotein, but also its unglycosylated precursors contain fatty acids indicating that glycosylation is not required for acylation.

To determine whether one or more of the MHV-A 59 glycoproteins are modified by acylation and at which step during virus maturation, infected Sac(−) cells were labeled with ^{3}H-palmitic acid from 7 to 8 hours post infection. Confluent monolayers in 35-mm petri-dishes of 2×10^6 Sac(−) cells were infected with MHV-A 59 or VSV (the Indiana strain, obtained from Dr. S. Schlesinger, Washington University School of Medicin, St. Louis, MO) using 30 or 10 PFU per cell, respectively. For labeling experiments with ^{35}S-methionine, the medium was removed and replaced by 1 ml methionine-deficient minimal essential medium supplemented with nonessential amino acids and with 5 per cent dialyzed fetal calf serum, 100 IU penicillin, 100 µg streptomycin and 25 µCi ^{35}S-methionine (1000 Ci/mmol; The Radiochemical Centre, Amersham, England). For labeling with ^{3}H-palmitic acid, 100 µCi of 9, 10-^{3}H(N)-palmitic acid (15.2 Ci/mmol; New England Nuclear Corp.) in 80 per cent ethanol was dried in a glass tube and taken up in 1 ml minimal essential medium containing 5 per cent fetal calf serum; this mixture was added to the cells. After the labeling period the cells were washed twice with PBS (4° C), lysed and the viral proteins were immunoprecipitated as previously described (6) with mouse anti-MHV-A 59 (16) or rabbit anti-VSV antiserum (4). Immunoprecipitates were analysed by electrophoresis in 15 per cent polyacrylamide gels. As previously reported, there is only one detectable ^{3}H-palmitic acid labeled polypeptide found in VSV-infected cells (14, Fig. 1 A). Also VSV, directly precipitated from infectious medium with polyethyleneglycol (PEG), revealed that only the G-protein contained fatty acids (results not shown). Analysis of proteins immunoprecipitated from cell lysates with mouse anti-MHV-A 59 anti-serum showed that fatty acid label

is present in a 150 kd and a 90 kd polypeptide (Fig. 1 B). The nucleocapsid protein contained no radioactivity and only trace amounts were detectable in the E 1-glycoprotein. The latter ^{3}H-label may have been due to conversion into metabolites other than fatty acids, which are incorporated into E 1 as suggested earlier (12). MHV-A 59 virions directly precipitated from the growth medium with PEG revealed that only the 90 K protein contained labeled fatty acid (results not shown). These results show that both intracellular forms of the E 2 glycoprotein (gp 90 and gp 150) are acylated. Attempts to block fatty acid acylation of the E 2 protein with cerulenin, as described by Schlesinger and Malfer (10), have not been successful.

Labeling of the E 2 and its precursor are not due to noncovalent interactions since these proteins were immunoprecipitated with anti-serum in the presence of 0.5 per cent Triton X-100 and 0.5 per cent 1,5-naphthalene-disulfonate-disodium salt. However, to obtain additional evidence that the

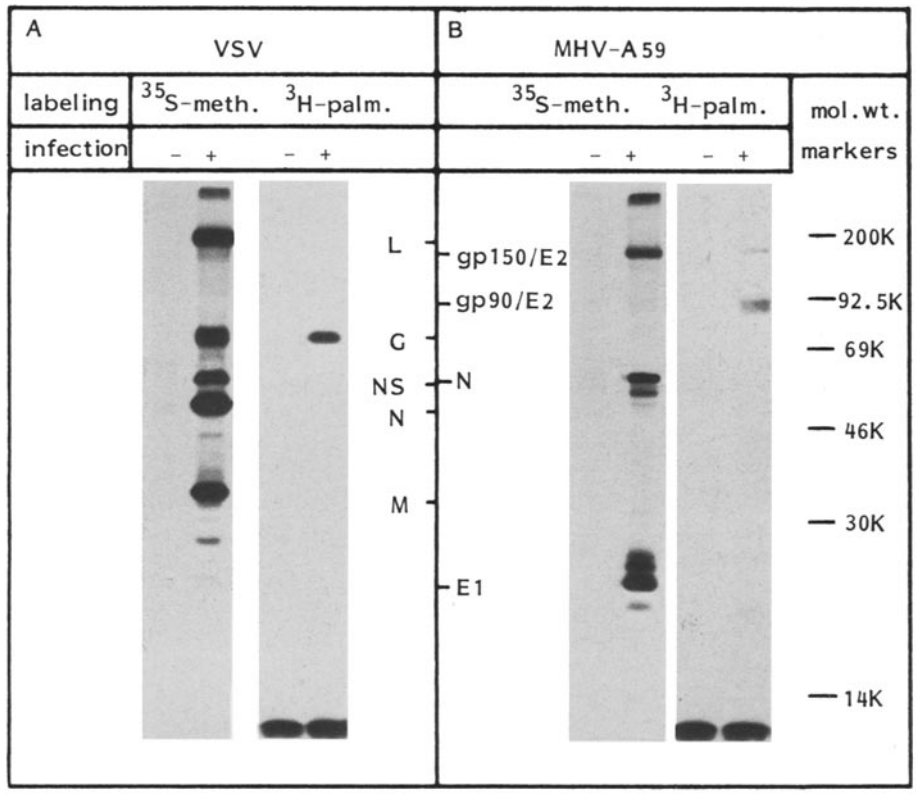

Fig. 1. Virus-specific proteins in MHV-A 59 and VSV infected cells. Proteins in MHV-A 59 or VSV infected and mock-infected Sac(−) cells were labeled with ^{35}S-methionine or ^{3}H-palmitic acid from 7 to 8 hours post infection. The virus-specific proteins in cell lysates were immunoprecipitated and analysed by polyacrylamide gel electrophoresis. The molecular weights of virus specific polypeptides were calculated using ^{14}C-methylated calibration proteins (Amersham)

palmitate label was covalently associated with E 2 and its precursor, SDS-polyacrylamide slab gels were treated with 1 M hydroxylamine (pH 6.6) for 16 hours prior to fluorography as described by Schlesinger et al. (11) and Bishr Omary and Trowbridge (3). Most of the label was removed when the E 2-glycoprotein labeled with ^{3}H-palmitic acid was exposed to hydroxylamine (results not shown). On the other hand, no detectable loss of radioactivity was observed when the ^{35}S-methionine labeled E 2 was treated with hydroxylamine. Similar results were obtained with VSV where palmitic acid is covalently bound to the G-protein (14).

To investigate more precisely which E 2-species of E 2 of MHV-A 59 are acylated, we followed the acylation in a pulse-chase experiment. Infected cells, incubated from 3 hours p.i. with or without 0.5 mg/ml tunicamycin were pulse-labeled at 7 hours p.i. for 15 minutes with ^{3}H-palmitic acid or ^{35}S-

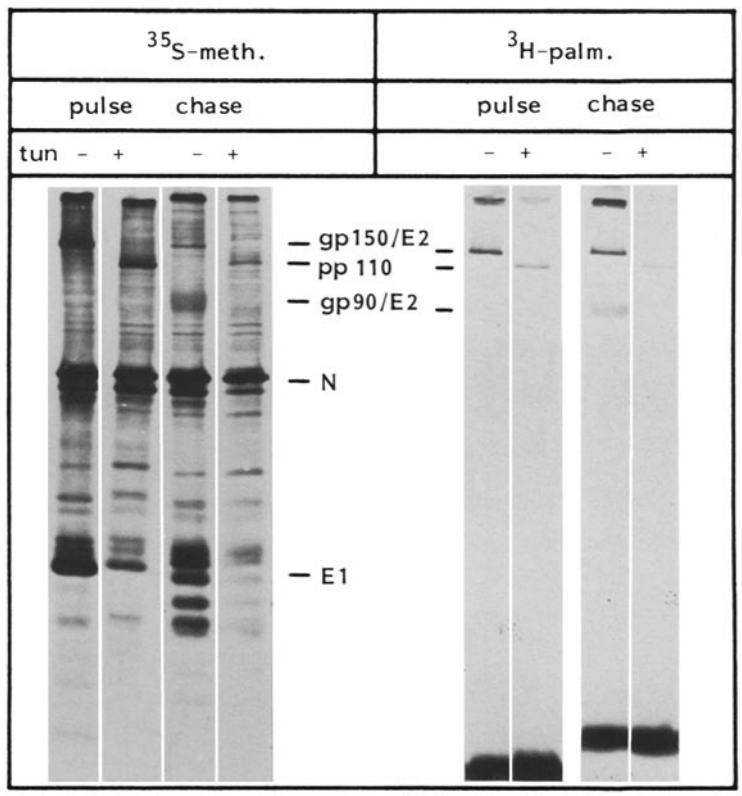

Fig. 2. Pulse-chase labeling and effect of tunicamycin of intracellular MHV-A 59 specific proteins. MHV-A 59 infected Sac(−) cells were pulse-labeled with ^{35}S-methionine or ^{3}H-palmitic acid for 15 minutes at 7 hours p.i. and chased for two hours in the absence (−) or in the presence (+) of 0.5 μg/ml tunicamycin *(tun)*; when used, this antibiotic was present from 3 hours post infection. Immunoprecipitates from the cell lysates were prepared and analysed by electrophoresis in 15 per cent polyacrylamide gels

methionine and the label was subsequently chased for two hours. Electrophoresis of the immunoprecipitates derived from ^{35}S-methionine pulsed cells produced the known set of virus-specific proteins (Fig. 2). In ^{3}H-palmitic labeled cells only gp 150 was detected. After the chase period the 90 K protein was detectable both in ^{35}S-methionine and ^{3}H-palmitic acid labeled cells. In the ^{35}S-methionine pulse-labeled MHV-A 59 infected cells grown in the presence of tunicamycin, all virus-specific proteins appeared with the exception of gp 150; instead the unglycosylated apoprotein with a mol. wt. of about 110 K was observed. This protein was also labeled with ^{3}H-palmitic acid and persisted even after a 2 hours chase period; no processing was observed.

Studies with Sindbis virus (15), ts mutants of VSV and tunicamycin-treated VSV-infected cells (13) suggest that acylation of the glycoproteins of these viruses occurs late during maturation, after glycosylation but prior to their movement to the cell surface. On the other hand, the Orsay strain of VSV grown at 30° C in the presence of tunicamycin, produces unglycosylated but acylated G protein (Go) (13). The significance of these two different kinetics of acylation in VSV G-protein is not clear at present. It has been shown earlier that tunicamycin-treatment of MHV-A 59 infected cells reduces the yields of extracellular infectious virus by more than 99 per cent (9). Viral particles continued to be released, but these particles are deficient in E 2 (5, 7). Repp et al. (8) showed that the E 2 antigen in tunicamycin treated cells stayed in perinuclear regions and within the rough endoplasmic reticulum. Glycosylation therefore plays a dominant role in transport of E 2 through the cell. Our results show that the acylation of unglycosylated glycoprotein of the VSV/strain Orsay is not an isolated anomaly. Also the unglycosylated 110 kd apoprotein of the intracellular E 2 precursor of MHV-A 59 is acylated; the pulse-chase experiments not only confirm that fatty acids can be added to the protein backbone itself, but also suggest that acylation of unglycosylated viral proteins does not restore the ability of these proteins to be transported through the cell.

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