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ANALYSIS AND LOCALISATION
OF MOUSE HEPATITIS VIRUS 3 (MHV3) POLYPEPTIDES

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RÉSUMÉ

ANALYSE ET LOCALISATION
des polypeptides du MHV3

L’analyse en gel de polyacrylamide des protéines de structure du
virus de l’hépatite murine type III permet de mettre en évidence cinq poly-
peptides dont trois sont glycosylés. Leurs poids moléculaires sont de
190 000 (VGPI50), 100 000 (VGP100), 58 000 (VP58), 25 000 (VP25), 24 000 dal-
tons (VGP24). L’analyse de sous-particules obtenues après un traitement
par une enzyme protéolytique, la bromélaïne, indique que VGP150 et
VGP100 sont les éléments constitutifs des spicules, que VP25 et VGP24
sont associés à l’enveloppe virale et que VP58 est le polypeptide consti-
tutif de la ribonucléoprotéine virale.

MOTS-CLÉS : Virus 3 de l’hépatite de la souris, Polypeptide ; Locali-
sation.

INTRODUCTION

Coronaviruses are morphologically distinctive RNA viruses which
have been classified in a separate taxonomic group [13]. While corona-
viruses have been extensively studied morphologically, they have not
been well-characterized chemically. An analysis of the structural compo-
nents of murine coronavirus MHV3 is in progress in our laboratory. This
report presents preliminary results concerning the analysis and the loca-
lisation of the MHV3 polypeptides.

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MATERIALS AND METHODS

The American Type Collection strain of MHV3 was grown and titrated as previously described on cells of the DBT line originating from a murine brain tumour [18, 19]. In order to obtain purified radioactively labelled virus, MHV3 was grown either in the presence of 1 µCi/ml 14C-L-amino acids (40 mCi/mM of carbon; CEA, France), or in the presence of 5 µCi/ml glucosamine 1H-1-hydrochloride (15 Ci/mMole; CEA, France) and 5 µCi/ml 3H-6-fucose (15 Ci/mMole; CEA, France). Fourteen hours after infection, the tissue culture fluid was removed and clarified by low speed centrifugation. Virus in the supernatant was precipitated by incubation with polyethylene glycol 6000 (PEG 6000) at a concentration of 8% (w/v) for 5 h at 4°C. The precipitate was dissolved in TNE buffer (NaCl 0.15 M, EDTA 0.005 M, 0.02 M Tris pH 7.2), layered on a 20-60% (w/v) sucrose density gradient in TNE buffer, and centrifuged for 1 h at 31,000 rpm in a Spinco SW41 rotor. The diffuse band in the upper half of the gradient was collected, diluted in TNE buffer and concentrated on a 90% glycerol cushion (35,000 rpm, 4 h, in a SW41 rotor). The concentrated virus was then layered on a positive density/negative viscosity glycerol/tartrate gradient (0-35% w/v potassium tartrate; 30% to 0% w/v glycerol in TNE buffer) and centrifuged 16 h at 31,000 rpm. The virus band was collected by aspiration. Bromelain (E. C.3.4.22.4; Sigma Chemicals) treatment of the purified virus was performed at a concentration of 1 mg/ml for 2 h at 37°C in TNE buffer in the presence of 0.1 mM mercaptoethanol. The treated virus was then repurified on a glycerol/tartrate gradient. For sodium dodecyl sulfate-polyacrylamide-gel electrophoresis (SDS-PAGE), the purified virus was dissolved in Tris-HCl 0.05 M pH 6.8, SDS 2%, β-mercaptoethanol 2%, glycerol 15% and bromophenol blue 0.001%. The samples were submitted to electrophoresis on slab gels (5% acrylamide stacking, 10% acrylamide resolving gels) according to the techniques described by Laemmli [7] and by Studier [14]. After electrophoresis, the labelled protein bands were visualized by the fluorographic method of Bonner and Laskey [2]. *Escherichia coli* RNA-polymerase subunits β (165,000 daltons) and β’ (155,000), phosphorylase b (94,000), bovine serum albumin (45,000), chymotrypsinogen (25,700), trypsin inhibitor from soybean (21,500) and cytochrome c (11,700), were used as standards for the molecular weight (MW) estimation of the viral polypeptides.

For electron microscopical observations, the viral particles were negatively stained by 2% sodium-phosphotungstate at pH 7 and examined in a Philips EM300 electron microscope.

RESULTS AND DISCUSSION

After concentration of the tissue culture fluid by PEG 6000, MHV3 was purified successively on a rate zonal sucrose gradient and on a density glycerol/tartrate gradient. In this density gradient, MHV3 sedimented in a homogeneous band at a density of 1.185 (data not shown). Typical coronavirus particles with a diameter in the range of 70 to 120 nm covered with surface projections 15 to 22 nm in length could be observed (fig. 1a). Separation of the polypeptides by SDS-PAGE of 14C-L-amino acid

MHV3 = mouse hepatitis virus 3.
MW = molecular weight.
PEG = polyethylene glycol.

VGP = virus glycoprotein.
VP = virus protein.
labelled MHV3 revealed the presence of five polypeptides (fig. 2a). By the same technique, but using $^3$H-glucosamine- and $^3$H-fucose-labelled virus, three of them were shown to contain carbohydrate (fig. 2b). The characteristics of the MHV3 polypeptides are: a glycoprotein of 190,000 daltons (VGP$_{190}$), a glycoprotein of 100,000 (VGP$_{100}$), a protein of 58,000 (VP$_{58}$), a protein of 25,000 (VP$_{25}$) and a glycoprotein of 24,000 daltons (VGP$_{24}$). Intact virus was treated with a proteolytic agent to identify those proteins which are exposed on the surface of the virus envelope. After treatment with bromelain, the surface projections could be entirely removed (fig. 1b). The despiked particles lost their infectivity and were less dense (1.16 in potassium tartrate) (data not shown). The bromelain treatment (fig. 2c) produced a complete loss of all the original polypeptides except the non-glycosylated polypeptide VP$_{38}$ which represents probably an inner core protein. The remaining polypeptides are membrane-associated; VGP$_{190}$ and VGP$_{100}$ were completely destroyed by the proteolytic agent and thus appear as constitutive of the surface projections. After bromelain treatment, a polypeptide of 18,000 daltons which could not be detected in the intact particles appears (fig. 2c). Densitometric analysis of different fluorographs (data not shown) revealed that in all cases the label of this neoproteins was approximatively the same that the label of VGP$_{24}$ and was significantly higher that the label of VP$_{25}$. Consequently, the 18,000-dalton protein seems derived mostly from VGP$_{24}$ and represents the portion of VGP$_{24}$ localised inside the lipid bilayers of the membrane. Thus VGP$_{24}$ was partially concealed within the viral envelope, and 25 % of this polypeptide was accessible to the enzyme. VP$_{25}$ which disappears after bromelain treatment has not been localised on the viral membrane. Although, this 18,000-dalton protein appears to be derived in great part from VGP$_{24}$, it is impossible to determine if it represents a single species of protein or if it also contains a polypeptide of similar MW issued from VP$_{25}$. Consequently, our results cannot indicate whether VP$_{25}$ is completely, digested by bromelain as in the case for VGP$_{190}$ and VGP$_{10}$, and thus appears localised at the surface of the membrane or whether VP$_{25}$, as VGP$_{24}$ is partially concealed in the viral envelope.
The available data on the structural proteins of coronaviruses from different species reveal some variety in number and size [1, 3, 4, 5, 6, 8, 9, 10, 11, 12, 15, 16, 20]. In general, the particle contains four to seven proteins. All reports indicate the presence of a major non-glycosylated protein of 50 to 60,000 daltons located inside the virion. One or two polypeptides below 50,000 daltons have been found, and at least one of these is glycosylated. A portion of these low MW proteins is localised inside the lipid bilayer. The polypeptides of high MW (2 to 4) are all glycosylated and involved in forming the surface projections.

SUMMARY

The analysis by SDS-polyacrylamide gel electrophoresis of purified mouse hepatitis virus type 3 revealed the presence of five polypeptides,
three of them contained carbohydrate. The molecular weight of these proteins was 190,000 daltons (VGP₁₁₀), 100,000 (VGP₁₀₀), 58,000 (VP₅₈), 25,000 (VP₂₅) and 24,000 (VGP₂₄). The analysis of particles obtained after limited proteolysis with bromelain suggests that VGP₁₁₀ and VGP₁₀₀ form the spike layer, that VP₂₅ and VGP₂₄ are membrane-associated and that VP₅₈ represents the inner core polypeptide.

Key-words: Mouse hepatitis virus 3, Polypeptide; Localisation.

Acknowledgments

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