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Differences in antigenicity of E2 in Semliki Forest virus particles and in infected cells

Brief Report

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Summary. Using six monoclonal antibodies to epitopes a–f on the glycoprotein E2 of Semliki Forest virus (SFV) we found antigenic differences between E2 in infected cells and in virus particles, respectively, if glycosylation was impaired by 2-deoxy-D-glucose or inhibited by N-methyl-1-deoxynojirimycin. Furthermore we concluded that a conformational change of E2 takes place on virus budding.

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The composition and structure of carbohydrate side chains attached to viral glycoproteins is important for virus maturation. They may influence virus adsorption and penetration, transport of viral glycoproteins [12], proteolytic cleavage of precursor proteins [1, 7], association of spike glycoproteins [13] or their antigenicities [5, 11]. With Semliki Forest virus (SFV), a togavirus containing the nucleocapsid protein C and three envelope glycoproteins E1, E2 and E3, we previously observed that virus maturation could be inhibited by impairment of glycosylation, e.g. by treatment of infected cells with tunicamycin or 2-deoxy-D-glucose (2-DG) [5]. Using polyclonal antisera, we have shown in addition that antigenicities of glycoproteins with no carbohydrate side chains (after treatment with tunicamycin) or with truncated chains (after treatment with 2-DG) differed from those of virus particles [2, 4, 9]. Using six monoclonal antibodies (mabs) specific for six epitopes a–f on virion E2 we recently found changes in antigenicity of virion E2, if SFV was propagated in cells in presence of N-methyl-1-deoxynojirimycin (MdN): released, infectious virus particles contained mannose-rich carbohydrate side chains of the composition $Glc_3Man_{7,8,9}(GlcNAc)_2$; epitope d reacted very well, epitopes c and e reacted less, and epitopes a, b and f only very slightly [6, 10].

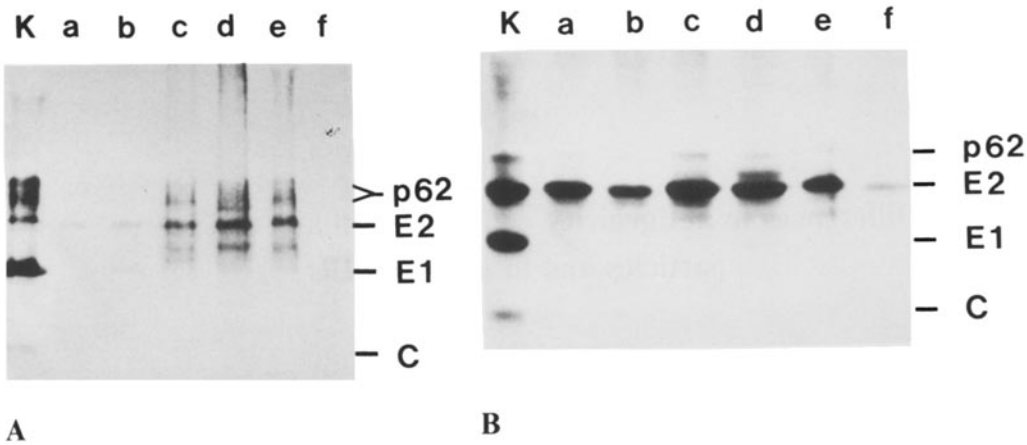

Fig. 1. Reactivities of the six mabs to epitopes *a–f* on E2 in SFV infected cells under conditions of radioimmunoprecipitation: CEC were infected with SFV in the presence of 5 mM 2-DG (**A**) or 2 mM MdN (**B**), and were metabolically labeled with ^{35}S-methionine. In addition samples of the respective lysates were treated with the rabbit antiserum *K*, which precipitates all structural components of the virion. Immunocomplexes were removed with protein A-containing *Staphylococcus aureus* cell walls. After SDS-PAGE (under non-reducing conditions) radioactivity was detected by fluorography. Note that in **A** additional precipitated bands (*c*, *d*, *e* bands between p62 and E2, and between E1 and E2, respectively) are due to different numbers of attached carbohydrate side chains on E2 or p62, respectively

Here we report results obtained in reactions of viral glycoproteins with the same mabs, but in infected cells. Briefly, chicken embryo cells (CEC) were infected with SFV at a moi = 100. The culture medium (MEM with 5% glucose) contained either 5 mM 2-DG, or 2 mM MdN, or no additions. 5.5 h p.i. viral proteins were labeled for 30 min with 25 µCi ^{35}S-methionine (Amersham Buchler)/2 ml/plastic Petri dish. Lysates were prepared for radioimmunoprecipitation (RIP) as described previously [6] and analyzed with the mabs or with a polyclonal rabbit antiserum K which precipitated all structural SFV proteins. Immune-complexes were subjected to SDS-PAGE according to Laemmli [8] under non-reducing conditions for separating E1 and E2 [6]. Proteins were detected by fluorography as described previously [6].

After treatment of SFV-infected cells with 2-DG, only epitopes c, d and e were accessible on E2 and on p62 (p62 is the intracellular precursor of E2 and E3) (Fig. 1A). The same result was obtained after treatment with tunicamycin (not shown). The results indicate that the structure of p62 and of intracellular E2, respectively, differed from that of correct glycosylated proteins. These observations are in agreement with our results obtained previously that sugar-free apoforms of p62 exposed antigenic sites absent in the virion [4].

If the first step of the oligosaccharide processing was blocked by MdN, all epitopes, except f, were accessible (Fig. 1B). In control infected cells, all epitopes were accessible (not shown). So epitope f only was recognized on E2 with complex carbohydrate side chains, as postulated previously [6]. But it was

interesting to observe that the pattern of accessible epitopes after treatment with MdN was not identical for intracellular viral glycoproteins and for E2 of the virion. These results can be explained by a structural and antigenic change occurring during the virus budding process. It indicates that composition of attached carbohydrate side chains on E2 influence virus antigenicity and is important for antibody reactivities. Other authors also reported that, if HIV-infected cells were treated with MdN, the accessibility of virion proteins to mabs was altered as well as bio-activity and conformations [2].

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