VP2 is the major exposed protein on orbiviruses

Brief Report

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Summary. Iodination of African horsesickness virus and epizootic hemorrhagic disease virus resulted in labeling of VP2 and not of the other capsid protein, VP5, suggesting that VP2 is the major surface exposed protein of these orbiviruses.

The outer capsids of all orbiviruses studied to date are composed of two proteins, VP2 and VP5 [14]. In bluetongue virus (BTV), there is evidence that VP2 is surface exposed to a much greater extent than VP5, since (1) VP2 is cleaved by proteases [17], (2) contains all the identified neutralizing epitopes [3, 4, 6, 12, 15], and (3) is the only protein that is selectively radiolabeled with an amino labeling reagent [9].

Herein, we have surface labeled two orbiviruses, African horsesickness virus (AHSV) and epizootic hemorrhagic disease virus (EHDV), to determine whether VP2 is also the major exposed protein in these viruses.

Low passage AHSV (serotype 7) and EHDV (serotype 2) were grown in Vero cells and purified as described by Mecham et al. [11], except that heparin was added to reduce adherence of cellular debris [5]. The proteins of partially purified EHDV and AHSV were resolved by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) [7, 9] and are shown in Fig. 1 A. As reported by others [1, 5], VP2, VP3, VP5, and VP7 are the major viral structural proteins.

EHDV and AHSV virions were radiolabeled using iodinated S-SHPP (sulfo-succinimidyl-3-[4-hydroxyphenyl]propionate) which reacts with ε-amino groups of lysine [16]. In agreement with the data from BTV [9], VP2 was the major iodinated protein in both viruses and virtually no VP5 was labeled.
Fig. 1. Surface labeling of EHDV and AHSV proteins. A Silver stain [18] of partially purified EHDV and AHSV proteins separated by 10% SDS-PAGE. Viral proteins were identified by comparison with $^{35}$S methionine labeled viral proteins (not shown). Molecular weight standards, in kilodaltons, were β-galactosidase, phosphorylase b, bovine serum albumin, and ovalbumin, respectively. B Fluorogram of EHDV and AHSV surface labeled with either S-SHPP, HPG or I$^+$. Proteins were separated by 10% SDS-PAGE (Fig. 1 B; the same results were obtained with AHSV serotype 3, data not shown).

After solubilization of viral proteins (in 150 mM NaCl, pH 6.0, followed by neutralization) VP5 from both viruses was readily labeled indicating that lysine residues are accessible on this protein when it is not enclosed in the virion (data not shown). The consistent exposure to solvent of the ε-amino group of lysine residues in proteins [8] makes reagents that label this group the most likely to correctly reflect surface exposure of a protein. Although the predominance of VP2 labeling with S-SHPP is clear, lack of surface labeling with a particular reagent can only suggest that a protein is not exposed. Therefore, to provide additional evidence that VP5 is not a major surface protein, virions were surface radiolabeled using the arginine specific reagent HPG (p-hydroxyphenylglyoxal) [19]. HPG was iodinated using $^{125}$I Na and N-bromosuccinimide [13] and reacted with EHDV and AHSV. In agreement with the results obtained with S-SHPP, arginine labeling iodinated predominantly VP2 in both viruses (Fig. 1 B). It should be noted that HPG was much less efficient in labeling virions than S-SHPP.

Iodination using electrophilic iodine (I$^+$) is not expected to accurately reflect polypeptide accessibility, since the potentially reactive carbons of tyrosine are...