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Minimal capsid composition of infectious human astrovirus

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ABSTRACT

Human astrovirus is an important etiological agent of acute gastroenteritis in young children. Despite advances in the characterization of the structure of the virion by cryo-electron microscopy and of capsid proteins by x-ray crystallography, the definition of the minimal polypeptide composition of infectious virus particles has been elusive. In this work we show that mature infectious particles are composed by only two proteins; VP34 that forms the core domain of the virus, and VP27 that constitutes the 30 dimeric spikes present on the virus surface. Our results also indicate that during the transition of immature (90 spikes) to mature (30 spikes) virus particles, that occur during trypsin activation, the viral protein VP25, that most likely forms the 60 spikes that are lost during maturation, detaches from the virus particle. This information is relevant to better understand the biology of virus entry and also for the efficient development of subunit vaccines.

1. Introduction

Human astroviruses (HAstVs) are common etiological agents of acute gastroenteritis in children, the elderly, and immunocompromised patients (Bosch et al., 2014; Cortez et al., 2017; Méndez, 2013). It is estimated that they are responsible for about 2–9% of acute, non-bacterial childhood diarrhea episodes (Vu et al., 2017), and a recent multicenter study showed that these viruses, together with norovirus and rotavirus exhibited the highest attributable burdens of diarrhea in the first two years of life (Platts-Mills et al., 2015). HAstVs are classified into 8 classical serotypes (HAstV-1 to −8) associated to acute gastroenteritis; however, novel HAstV strains have been recently described, which have also been associated with neurological disease, including meningitis and encephalitis in immunocompromised patients (Vu et al., 2017).

HAstVs are nonenveloped particles constituted by a genome of about 6.8 kb, surrounded by a capsid with a T = 3 icosahedral symmetry. The capsid is initially assembled intracellularly from a polyprotein precursor of about 90 kDa. During the exit of the virion from the cell, the virus particle is cleaved by caspases to yield a virus formed by a 70 kDa protein (VP70) (Arias and DuBois, 2017). The VP70-containing, still immature virus capsid, is further processed by extracellular proteases; *in vitro,* infectious particles can be obtained by treatment of the virus with trypsin. This treatment has been shown to increase the infectivity of HAstV 10^3 to 10^5-fold (Bass and Qiu, 2000; Mendez et al., 2002; Sanchez-Fauquier et al., 1994). The size and number of polypeptides that constitute the infectious HAstV after protease processing is still not completely defined. There are conflicting reports that suggest that the infectious virions are composed by two to six proteins in the range of 5.5–42 kDa (Greenberg and Matsui, 1992; Willcocks and Madeley, 1992). However, more recently a consensus has emerged suggesting that infectious viruses are formed only by three polypeptides in the range of 32–34, 27–29, and 25–26 kDa (Bass and Upadhyayula, 1997; Belliot et al., 1997; Mendez et al., 2002; Sanchez-Fauquier et al., 1994), depending on the virus strain.

In this work, we have probed the structure of infectious HAstV through exhaustive trypsin digestion of purified particles and Western blot analysis of the polypeptides that form the virus capsid. It is relevant to know the precise structural composition of the infectious virus to learn about the biology of virus entry and also for the efficient development of subunit and virus-like particles vaccines.

2. Material and methods

HAstV-8, strain Yuc8, was grown in Caco-2 cells as described (Mendez-Toss et al., 2000), except that 200 μg/ml of trypsin were used to activate virus infectivity. The protocol for purification of the virus is based on a procedure previously reported, and is described in detail below (Sanchez-Fauquier et al., 1994). The infectivity of the virus was determined by an immunoperoxidase focus-forming assay, as described (Mendez et al., 2004). For Western blot analysis, the viral proteins were resolved in 10% SDS-polyacrylamide gels, transferred to nitrocellulose membranes and incubated with a hyperimmune serum to Yuc8, as previously reported (Mendez et al., 2004). Silver staining of gels was

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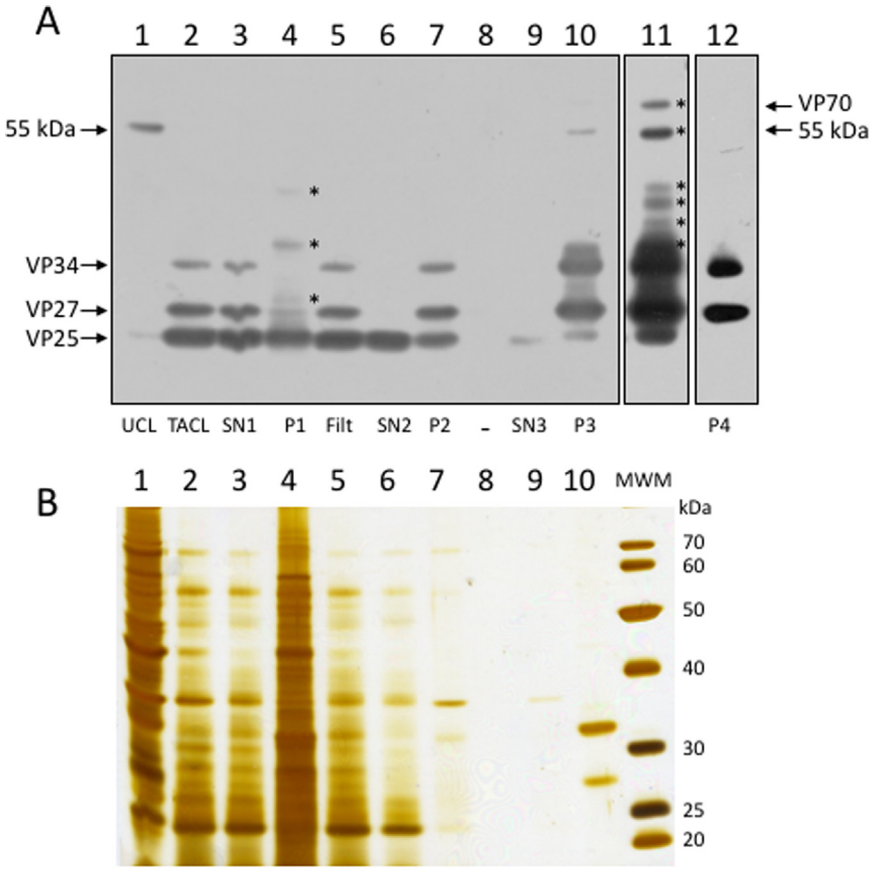

Fig. 1. Protein composition of mature, infectious human astrovirus. Different fractions of the purification protocol of HAstV-8 from infected Caco-2 cell lysates, analyzed either by Western blot using a hyperimmune polyclonal serum to purified HAstV-8 particles (panel A), or by silver-staining (panel B) of the proteins separated in an 10% SDS-gel. 1) untreated infected cell lysate (UTCL); 2) trypsin-activated infected cell lysate (TACL); 3) supernatant of clarification step (SN1); 4) pellet of clarification step (P1); 5) filtered supernatant of clarification step (Filt); 6) supernatant of ultracentrifugation step (SN2); 7) pellet of ultracentrifugation step (P2); 8) empty lane; 9) supernatant of ultracentrifugation through a sucrose cushion (SN3); 10) pellet of ultracentrifugation through a sucrose cushion (P3); 11) overexposure of lane 10; 12) pellet of ultracentrifugation through a second sucrose cushion after further digestion of the sample with trypsin (P4). Precursor structural proteins are indicated with an asterisk. The molecular weight markers (MW) are indicated. The analysis of fractions during the purification protocol was performed three times; a representative gel is shown. As reference, in both panels, the relative proportion of the total sample loaded for each sample in the gel, for samples in lanes 1–10 and in lane 12, was: 1; 1; 1; 5; 1; 1; 1.25; 1.25; 20; 33.

carried out using a standard procedure. ß-octylglucoside was from Pierce (cat. # 28310); trypsin was from Gibco (cat. # 27250–018)

3. Results and discussion

As model HAstV, in this work we used the strain Yuc8 of HAstV serotype 8, whose mature form has been reported to contain three protein species of approximately 34 (VP34), 27 (VP27), and 25 (VP25) kDa (Arias and DuBois, 2017). During the purification procedure, we followed the viral proteins present in the various fractions by Western blot analysis, using polyclonal antibodies raised against purified HAstV-8 particles (Fig. 1A), or by silver-staining (Fig. 1B). The infectivity of the virus present in each fraction was also determined by an immunoperoxidase focus-forming assay (Fig. 2).

For the purification procedure, confluent monolayers of Caco-2 cells were infected with HAstV-8 at an MOI of 1, and the cells were incubated for 18 h at 37 °C. After this time, the cells were frozen and thawed three times, and the resulting cell lysate was analyzed by Western blot before and after treatment with 200 µg/ml of trypsin for 1 h at 37 °C. Before trypsin treatment, a single band of about 55 kDa was detected, most likely corresponding to an intermediate cleavage product of the viral protein VP70 (Fig. 1A, lane 1). In the protease-treated cell lysate, the 55 kDa band disappeared and three bands of 34, 27, and 25 kDa were observed, which correspond to the reported capsid core (VP34) and the spike VP27 and VP25 proteins (Fig. 1A, lane 2).

The trypsin-treated lysate was subsequently clarified by centrifugation at 2000 × *g* for 10 min at 4 °C, and the supernatant and the pellet of this centrifugation were analyzed (Fig. 1A, lanes 3 and 4, respectively). As expected, the supernatant showed the same protein pattern as the trypsin-activated total cell lysate (compare lanes 2 and 3 in Fig. 1A), since the viral particles do not sediment under these conditions. Of interest, in the pellet of the clarification step only traces of the mature viral capsid protein VP27 were found, while VP25 and putative precursors of the mature structural proteins were observed; these proteins are probably bound to cell membranes, since they pelleted at low speed centrifugation (Fig. 1A, lane 4). In agreement with these observations, most of the infectious virus remained in the supernatant, while the pellet did not contain infectious particles (Fig. 2). The supernatant of the previous step was then filtered through a 0.45 µm nitrocellulose membrane (Fig. 1A, lane 5), and the clarified and filtered cell lysate was ultracentrifuged at 50,000 × *g* during 16 h at 4 °C; the resulting supernatant and pellet of this centrifugation were again characterized (Fig. 1A, lanes 6 and 7). Of interest, while essentially all of the infectious viral particles, constituted by VP34, VP27, and VP25, sedimented under these conditions (Fig. 2, lane P2), a fair amount of VP25 was detected in the supernatant (Fig. 1A, lane 6), probably as result of this protein being released from the virions during the ultracentrifugation step. In agreement with this possibility, the VP27/VP25 ratio was found to be increased in the virus present in the pellet of this centrifugation, as compared to that observed in the trypsin-treated as well as in the clarified and filtered cell lysate (Fig. 1A, compare lanes 2 and 5, with lane 7). However, it is also likely that VP25 is produced in large quantities during the trypsin treatment of whole cell lysate (lane 2), not necessarily as part of an infectious virion, and that no other step was sufficient to remove all VP25 that was not associated with the virion. Thus, some proportion of the VP25 protein found in the supernatant (lane 6) could represent still non-virus associated VP25 protein.

Finally, the pellet of the previous ultracentrifugation step was resuspended in 4 ml of 0.5% ß-octylglucoside in TNE buffer (50 mM Tris-HCl, pH 7.4, 0.1 M NaCl, 10 mM EDTA) and incubated for 30 min at 4 °C, to release the virus particles that could be associated with cellular membranes. This sample was then centrifuged through a 1 ml 30% sucrose cushion in TNE buffer, at 200,000 × *g* for 2 h at 4 °C. In the top fraction of the cushion that did not go through the sucrose fraction, remnants of VP25 were found (Fig. 1A, lane 9), while the infectious