Human Rhinovirus Subviral A-Particle binds to Lipid Membranes over a Twofold Axis of Icosahedral Symmetry

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Short title: Topology of rhinovirus A-particle membrane interaction

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Minor group human rhinoviruses bind LDL-receptors for endocytosis. Once inside endosomes, the acidic pH triggers their dissociation from the receptors and conversion into hydrophobic subviral A-particles; these attach to the membrane and transfer their ss(+)-RNA genome into the cytosol. Here, we allowed HRV2 A-particles, produced in vitro by incubation at pH 5.4, to attach to liposomes; cryo-electron microscopy 3-dimensional single-particle image reconstruction revealed that they bind to the membrane around a two-fold icosahedral symmetry axis.

HRV2, produced and purified as described (7), was incubated at pH 5.4 for 15 min at room temperature. This results in complete conversion of native virus into subviral (expanded full) A-particles with a sedimentation constant of 135S, as compared to 150S for native virus. We noticed that our viral preparations also contained variable proportions of natural empty capsids (NEC) also called ‘natural top component’. At pH ≤ 5.6 these are converted into expanded empty shells with diameter and sedimentation identical to the empty particles remaining after uncoating of native virions, except from containing VP0 instead of VP2 and VP4 (ref. (9) and our unpublished observations).

The material resulting from acidification was incubated with ~100 nm diameter unilamellar liposomes (2) for 30 min, applied to holey carbon grids, flash frozen, and viewed in a Polara cryo-electron microscope equipped with a CCD camera at detector magnification of 79,372 x (1.89 Å/pixel (px) sampling rate). Micrographs (as in Fig. 1A) were acquired at underfocus between 1.5 and 5.4 µm and single particles (239) were manually picked by using Xmipp-2.4 (12) and boxed (256 x 256 px) with RELION (16). The high defocus was used to increase the visibility of the contacts between virus and membrane with, at the same time, accepting the ensuing fringe-related artefacts. We found it impossible to ascertain that a particle entirely below or above a vesicle is definitely membrane-attached, therefore, only those images with a clear side-view of the lipid bilayer (that was about 5 nm in thickness) were selected. These included some particles seen to be attached to the circumference of the liposome (like in Fig. 1B, and 1C c, e) and above or below it (like in Fig. 1C a, b, d, f). Our preliminary electron cryo-tomography results ascertained that also such latter particles were definitely contacting the membrane and had not just accidentally became positioned close to it (unpublished results). The contrast transfer function (CTF) was estimated with ctfind3 (13); although this software is able to correct major and minor defocus values, micrographs with grossly astigmatic Thon rings were discarded. Because of the low number of individual particles per micrograph those with similar CTF were combined in ten groups for better noise.
estimation (15). CTF-corrected, contrast-inversed, and normalized particle images were subjected to three consecutive rounds of 2D-classification (16). During this process 15 particle images with obvious distortions segregated into separate groups and were discarded.

A 3D-reconstruction (3DR) was then achieved with RELION run to convergence on the final dataset of 224 single particle images, covering the defocus values indicated above, using a cryo-electron microscopy 3DR model of the HRV2 A-particle (manuscript submitted) filtered to 60 Å as starting map. In the first round, icosahedral (I2) symmetry was enforced using particle images masked with an (apodized) circular mask of diameter 330 Å, i.e. 175 px, leading to a 3DR of about 20 Å final resolution (Fig. 2A). This model (filtered to 60 Å) was then used as starting map for 3DR without imposing symmetry. Several 3DR were run using values for the particle mask diameter between 350 and 390 Å as to vary the contribution of the (asymmetric) liposomal membrane and the (symmetric subviral) particle to the final model. A mask diameter of 380 Å appeared to best reveal the icosahedral symmetry of the particle together with a distinct adhering membrane patch; it converged at a resolution of about 37 Å (Fig. 2B). Despite substantial distortion with respect to a perfect icosahedron and a cleft at the membrane-proximal side of the particle, the superposition of the symmetric (I2) 3DR and the asymmetric (C1) 3DR allowed for unequivocal identification of the symmetry axes of the icosahedral virion shell (Fig. 2C) and establishing their position with respect to the lipid bilayer. The membrane extended over one of the two-fold axes including two star-like mesas at the five-fold axes. This was confirmed by difference mapping, arithmetically subtracting the I2 3DR from the C1 3DR; the most important difference was again manifest just above one of the two-fold axes (not shown). The cleft (see arrow in Fig. 2E) is most probably an artefact resulting from fringes appearing at the high defocus values chosen for better visibility of the membrane contacts and/or from an unequal angular distribution of the (small number) of particle images.

Strauss and colleagues recently reported the 3D-structure of membrane-bound poliovirus A-particles solved by cryo-tomography (17). They triggered receptor-catalyzed conversion of virus pre-bound to Ni-NTA-lipid-containing liposomes carrying his-tagged soluble poliovirus receptor by incubation at 37°C (19). The ensuing A-particles are presumably handed over to the membrane where they were seen to interact with the lipid bilayer via elongated extensions close to a two-fold axis.

We here show that single particle reconstruction from 224 particles can reliably localize the site of interaction between the A-particle of HRV2, another member of the genus Enteroviruses, and a lipid membrane. Mixing A-particles, prepared by incubation of native
HRV2 at acidic pH to mimic physiologic conditions as within the endosome during infection, with liposomes resulted in direct attachment of the lipophilic particles to the lipid membrane (11). Per analogy to poliovirus, attachment likely occurs via the externalized amphiphilic N-terminal segments of VP1 (5) and can lead to RNA transfer through channels in the lipid bilayer (18). At least poliovirus A-particles were shown to be infectious, although with low efficiency, indicating their capacity to transfer RNA through a cellular membrane (2). Our reconstruction of the membrane-bound HRV2 A-particles does not show the ‘umbilical’ connections as reported for poliovirus A-particles (17). Rather, the protein shell was found to be in intimate contact with the membrane. This is clearly seen in the 3DR even despite the presence of the artefactual cleft most probably resulting from the high defocus. Why our data and those of poliovirus A-particles bound to liposomes differ is unclear; as stated by the authors, the ‘umbilici’ might be aggregates composed of extruded VP4, the N-terminal extensions of VP1 together with RNA and/or poliovirus receptor molecules. In our case, no receptors were present making the system cleaner and more easily interpretable. Our present data on HRV2 suggest that the N-terminal amphipathic segments of VP1 that become exposed between the tips of the three-bladed propellers at the three-fold symmetry axes and the ascent of the star-like mesa at the five-fold axes (3) and convey membrane attachment (5, 10), might ‘pull’ the protein shell towards the membrane. This would result in the establishment of a tight seal around the hole at the two-fold axis, where the RNA exits.

When cryo-EM images were taken from native HRV2 that had been preincubated with recombinant his6-tagged soluble very-low density lipoprotein receptor fragments (7) and exposed to Ni-NTA-lipid containing liposomes (1) 3DR demonstrated more prominent domes at the five-fold axes because of receptor-derived additional density (20). Furthermore, density stemming from the membrane above one up to three of the domes could be discerned (unpublished results); however, the distance from the virion centre to the membrane surface was longer when compared to subviral particles directly attached to the membrane. Thus, like in poliovirus (4), RNA passage from within receptor-bound virions through the lipid bilayer into the cytosol is unlikely. This agrees with older data (8) in that the virus has to leave the receptor and convert into A-particles that subsequently (or concomitantly) attach directly to the lipid bilayer of the endosome for RNA passage into the cytoplasm.

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**Figure Legends**

Fig. 1 Examples of cryo-electron micrographs of A-particles attached to liposomes. HRV2 was incubated for 15 min in 50 mM Na-acetate (pH 5.4) resulting in its conversion into A-particles that were subsequently incubated with a suspension of liposomes (~100 nm diameter) at pH 7.4 for 30 min. The sample was then applied onto holy carbon grids, vitrified with a Leica EM GP, and images were collected at 79,372 x magnification in a FEI Tecnai F30 Polara electron microscope with a Gatan Ultrascan 4000 4k CCD camera as in (6). A) Section of one of the micrographs (5.1 μm underfocus). B) HRV2 A-particles attached to a liposome within the same plane as seen from the equal distance of their centres from the membrane contacts C) Six examples of extracted (contrast-inversed) particle images (out of the 224 used in the 3DR). Particles are seen to be attached at the circumference of the vesicle (as in B; Cc, e) or below or above it (Ca, b, d, f). Note the strong negative density at the contact site between the A-particle and the membrane that is most probably an artefact resulting from fringes occurring at the high defocus. It might give rise to the cleft seen in the reconstructions (see arrow in Fig. 2E). Scale bars, 30 nm.

Fig. 2 HRV2 attaches to a lipid bilayer over a two-fold axis including two star-like mesas at five-fold axes. 3DR rendered with Chimera (14). A) Computed by imposing I2 symmetry, view down a 2-fold axis. B) Computed without imposing symmetry, same view as in A). C) Superposition of A) and B). D – F) as A – C) but turned by 90° around the x-axis. Images rendered at a contour level of ~2 sigma (I2 3DR) and ~1.7 sigma (C1 3DR). Colour gradients (distance from the particle centre) are 140 Å (white) to 150 Å (dark colours). For better orientation, an icosahedral mesh is displayed in yellow. Arrow points to a cleft that is
probably the product of fringes resulting from the high defocus values and the strong local changes in density at the contact points between viral protein and lipid membrane.

References


