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Single particle cryoelectron tomography characterization of the structure and structural variability of poliovirus–receptor–membrane complex at 30 Å resolution

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Abstract

As a long-term goal we want to use cryoelectron tomography to understand how non-enveloped viruses, such as picornaviruses, enter cells and translocate their genomes across membranes. To this end, we developed new image-processing tools using an *in vitro* system to model viral interactions with membranes. The complex of poliovirus with its membrane-bound receptors was reconstructed by averaging multiple sub-tomograms, thereby producing three-dimensional maps of surprisingly high-resolution (30 Å). Recognizable images of the complex could be produced by averaging as few as 20 copies. Additionally, model-free reconstructions of free poliovirus particles, clearly showing the major surface features, could be calculated from 60 virions. All calculations were designed to avoid artifacts caused by missing information typical for tomographic data (“missing wedge”). To investigate structural and conformational variability we applied a principal component analysis classification to specific regions. We show that the missing wedge causes a bias in classification, and that this bias can be minimized by supplementation with data from the Fourier transform of the averaged structure. After classifying images of the receptor into groups with high similarity, we were able to see differences in receptor density consistent with the known variability in receptor glycosylation.

Keywords: Cryoelectron tomography; Poliovirus; Cell entry; Virus–receptor–membrane complex

1. Introduction

Electron tomography is an emerging methodology that is capable of providing three-dimensional (3D) information of individual large cellular specimens (Baumeister, 2005; Frank, 2006; McIntosh et al., 2005). Traditionally, electron tomographic studies were done on biological samples that had been fixed, stained to improve contrast, and plastic-embedded for sectioning. Such studies have provided invaluable insights concerning the structure of cellular organelles and supramolecular complexes with irregular structures. Within the past few years there has been an increased focus on the use of electron tomography of samples embedded in vitreous ice. These low-dose cryoelectron tomography studies (as reviewed in Baumeister, 2005; Grunewald and Cyrklaff, 2006; Lucic et al., 2005; McIntosh et al., 2005; Subramaniam, 2005) originally emerged as a way to investigate different sorts of problems from those addressed by the more common single-particle cryo-EM reconstruction approaches. In conventional single particle methods, reconstructions from two-dimen-

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sional projections focus on systems where averaging over multiple copies of conformationally identical particles can produce images at relatively high (sub-nanometer) resolution (Cheng et al., 2004; Fotin et al., 2004; Ludtke et al., 2004). In contrast, early cryoelectron tomographic studies focused on rather thick, unique or conformationally diverse structures, and asked questions where much lower resolution information (>10 nm) was sufficient.

Recently a new class of problem has emerged that is well-suited to a combination of the two approaches: it combines the power of tomographic methods to derive three-dimensional structures of individual molecules with the improvement in signal-to-noise (and consequently resolution) provided by averaging over multiple similar structures (Walz et al., 1997). Initially, this approach was applied to determine the structures of macromolecular complexes *in vitro* (Beck et al., 2004; Cardone et al., 2007; Chang et al., 2007; Cheng et al., 2007; Deng et al., 2007; Grunewald et al., 2003; Harris et al., 2006; Nicastro et al., 2005, 2006; Nickell et al., 2007; Zanetti et al., 2007; Zhu et al., 2006). Perhaps more excitingly, the approach has begun to be used to study macromolecular complexes within the context of intact cells. The ultimate goal is to be able to interpret tomographic reconstructions of crowded cell interiors in terms of their smaller components by matching a library of known higher-resolution structures to features in the low-resolution landscape. Several recent papers have demonstrated substantial progress in this direction, showing that is possible to map individual particles in cells (Bohm et al., 2000; Frangakis et al., 2002; Garvalov et al., 2006; Medalia et al., 2002; Murphy et al., 2006; Ortiz et al., 2006), and in favorable cases to derive reconstructions of macromolecular complexes such as the ribosome, to a few nanometers resolution (Ortiz et al., 2006).

Studying the process of viral infection in cells is an exciting potential use of this technique, both because there has already been extensive work characterizing the structures of viruses and viral components in isolation, and because there is often internal symmetry in the component structures. This aids in the identification of entry intermediates within cells and assists in the production of reconstructions at significantly higher resolution than would be possible with asymmetric structures.

The process of viral entry by non-enveloped viruses is poorly understood. In contrast to enveloped viruses, where the fusion of an outer viral membrane with a cellular membrane provides a conceptually simple mechanism for delivering the viral genome into the cytoplasm, non-enveloped viruses face a more difficult topological problem: they must pass a large, highly charged nucleic acid genome or nucleoprotein complex across a lipid bilayer to gain access to the appropriate intracellular compartment for replication. Different models have been suggested for this entry process, such as disrupting the membrane, creating a pore, or co-opting the translocation machinery of its targeted host cells (Hogle, 2002).

We have focused on poliovirus as a particularly attractive model for understanding how non-enveloped viruses solve this topological problem. Poliovirus, a well-studied prototype member of the picornavirus family (Hogle, 2002), is a small, non-enveloped virus consisting primarily of a $T = 1$ icosahedral protein capsid, 30 nm in diameter, enclosing a 7500-nucleotide positive-sense RNA genome (Hogle et al., 1985). Poliovirus infection is initiated though binding of its receptor, called Pvr or CD155, a CAM-like molecule with three extracellular Ig-like domains, a transmembrane domain and a C-terminal cytoplasmic domain (Mendelsohn et al., 1989). At physiological temperature, receptor binding catalyzes a dramatic conformational rearrangement to form an altered particle called the 135S particle or A particle (Joklik and Darnell, 1961). This conformational rearrangement results in the externalization of a myristoylated capsid protein, VP4, and of the N-terminal extension of capsid protein VP1, both of which are located on the inner surface of the mature virion capsid (Fricks and Hogle, 1990). These two externalized peptides then insert into membranes. Electrophysiological experiments *in vitro* show that the externalized peptides can form channels and pores (Tosteson and Chow, 1997; Tosteson et al., 2004). Genetic experiments demonstrate that certain mutations can alter both the ability to form channels and the ability to release the viral genome during infection (Danthi et al., 2003). These results have led to the suggestion that the channels, or some related perturbation of the membrane, play an essential role, allowing the viral genome to cross cell membranes and to be delivered into the cytoplasm.

To better understand this process, we and others have used a combination of X-ray crystallography and cryoelectron microscopy to study the structures of soluble forms of several key cell entry intermediates of poliovirus, including the mature virions (160S particles), virions decorated with the ectodomains of host–cell-specific receptors (Belnap et al., 2000b), the 135S particles (Belnap et al., 2000a; Bubeck et al., 2005a), and the empty particles (80S particles) that result after loss of the viral genome (Belnap et al., 2000a). More recently, we have developed a simple model system for biochemical and structural characterization of membrane-associated cell entry intermediates. In this model membrane system, liposomes containing low levels of lipids with NTA head-groups are used to capture C-terminally His-tagged ectodomains of Pvr. These receptor-decorated liposomes have been shown to bind virus, to induce the transition to form 135S particles, and to result in the insertion of VP4 and the N-terminus of VP1 into membranes (Tuthill et al., 2006). Structural studies of the virus in complex with a membrane-anchored receptor (Bubeck et al., 2005b) demonstrate that poliovirus approaches the membrane along its fivefold axis and binds five copies of Pvr. The density in the reconstruction also suggests that receptor binding produces a distortion of the outer leaflet of the membrane, which is seen as an outward projection of the bilayer, occurring just below the fivefold peak on the viral surface (Bubeck et al., 2005b).