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Clathrin-Coated Vesicles from Brain Have Small Payloads: A Cryo-Electron Tomographic Study

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Abstract

Clathrin coats, which stabilize membrane curvature during endocytosis and vesicular trafficking, form highly polymorphic fullerene lattices. We used cryo-electron tomography to visualize coated particles in isolates from bovine brain. The particles range from ~66 to ~134 nm in diameter, and only 20% of them (all ~80 nm) contain vesicles. The remaining 80% are clathrin “baskets”, presumably artifactual assembly products. Polyhedral models were built for 54 distinct coat geometries. In true coated vesicles (CVs), most vesicles are offset to one side, leaving a crescent of interstitial space between the coat and the membrane for adaptor proteins and other components. The latter densities are fewer on the membrane-proximal side, which may represent the last part of the vesicle to bud off. A small number of densities - presumably cargo proteins - are associated with the interior surface of the vesicles. The clathrin coat, adaptor proteins, and vesicle membrane contribute almost all of the mass of a CV, with most cargoes accounting for only a few percent. The assembly of a CV therefore represents a massive biosynthetic effort to internalize a relatively diminutive payload. Such a high investment may be needed to overcome the resistance of membranes to high curvature.

Keywords

clathrin-mediated endocytosis; cryo-electron microscopy; three-dimensional image reconstruction; adaptor proteins; fullerenes

Introduction

Clathrin-mediated endocytosis is responsible for cellular uptake in the context of receptor recycling (LDL) (Ehrlich et al., 2004)), synaptic vesicle recycling (Augustine et al., 2006), virus infection (e.g. (Ehrlich et al., 2004; Matlin et al., 1981; Rust et al., 2004)), and import of the prion protein (Taylor et al., 2005), among other processes. Clathrin-coated vesicles are also involved in protein sorting at the trans-Golgi network (Traub, 2005), and the assembly of the Golgi apparatus itself requires clathrin (Radulescu et al., 2007). This

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functional diversity requires assembling polymorphic scaffolds that are able to accommodate large variations in the size, shape, and molecular nature of the cargoes.

The building-block - the clathrin triskelion - is a remarkable structure with three hinged 52 nm-long legs connected at a trimeric hub (Brodsky, 2012; Kocsis et al., 1991; Ungewickell and Branton, 1981). It is able to assemble into many different forms, including flat lattices (Heuser, 1989), clathrin baskets (CBs) which are proteinaceous particles devoid of lipid (Crowther and Pearse, 1981; Crowther et al., 1976; Pearse and Robinson, 1984; Vigers et al., 1986b), and clathrin-coated vesicles (CVs) (Crowther et al., 1976). The coats of CBs and CVs adopt a wide range of polyhedral shapes and sizes.

In CV assembly, the main role of clathrin is to impose curvature of the membrane or to stabilize curvature otherwise accomplished (Hinrichsen et al., 2006). This calls for an energetically unfavorable distortion of the lipid bilayer. The endocytic process starts with the formation of a coated pit, followed by deepening the invagination until it is pinched off from the membrane of origin as a CV through the action of the GTPase dynamin (Hinshaw, 2000). Once the CV detaches from the plasma membrane, it is rapidly uncoated by the ATPase, Hsc70, and the freed clathrin triskelions recycle back to the membrane. The lifetime of a CV is only a few seconds before it is uncoated (Taylor et al., 2011). In view of these kinetics, it is likely that biochemical isolates contain, in addition to *bona fide* CVs recently budded off, also CBs assembled in the homogenate and CVs completed from coated pits during the isolation procedure.

In CVs, the clathrin network is coupled to the vesicle membrane through various proteins, the major ones being the adaptor proteins (APs), AP-1 and AP-2, which also function in cargo selection (Edeling et al., 2006). Each AP is composed of a heterotetrameric complex, forming a four-chain core, with two chains extending from the core to form two appendages or “ears”. Various parts of the APs have been put forward as the clathrin-binding parts, such as the AP-2 core (Matsui and Kirchhausen, 1990; Peeler et al., 1993), the 1-appendage (Goodman and Keen, 1995), and the linker or hinge of the 2-appendage (Shih et al., 1995) at a motif called the clathrin box (Dell’Angelica et al., 1998; ter Haar et al., 2000). It remains unsettled which potential interactions are important in vivo.

The polymorphism of clathrin-coated particles complicates analyses of their three-dimensional structures by “single particle” reconstructions from cryo-electron microscopy. Classification of images into homogeneous subsets represents one approach to overcoming this obstacle and most studies to date have focused on the D6, 36-triskelion, coat which is relatively abundant in preparations from brain (Fotin et al., 2004; Heymann et al., 2005; Smith et al., 1998; Smith et al., 2004; Xing et al., 2010). However, this structure is a CB lacking a cargo and *per se* it casts little direct light on the wide range of clathrin lattices that form nor of the interactions of the clathrin coat with other components. The more recently introduced technique of cryo-electron tomography (cryo-ET) (Baumeister and Steven, 2000; McEwen and Frank, 2001) has the advantage of rendering three-dimensional structures for individual particles and its potentiality for investigating clathrin-coated particles has been demonstrated (Cheng et al., 2007). Here we have followed a generally similar approach, working with a larger data set and focusing to a greater extent on coat polymorphisms, their complements of APs, the presence of vesicles, and, in particular, the quantitation of the mass contributions of the respective constituents.

Materials and Methods

Preparation of clathrin-coated particles

Material was isolated from fresh bovine brains essentially following the procedure of Nandi et al. (1982) (Nandi et al., 1982) and using a 12% sucrose-D₂O ultracentrifugation step (SW28 rotor, 100,000xg for 3h) for purification. The preparation was stored at a protein concentration of about 5 mg/ml at 4°C in the homogenization buffer (0.1M MES, 0.1 mM EGTA, 0.5 mM MgCl₂, and 3 mM NaN₃, pH 6.5). Multiple preparations were done and used within a week (longer storage resulted in precipitation and freezing with cryo-protectants also failed).

Data acquisition

The specimen was mixed with an equal volume of 10 nm colloidal gold particles to serve as fiducial markers (BBInternational, Ltd), giving $\sim 3 \times 10^{12}$ particles/ml, applied to a glow-discharged lacy carbon grid and plunge-frozen (Reichert Jung KF80 Cryofixation System). The SerialEM package (Mastronarde, 2005) was used to record tilt series on a Tecnai T12 electron microscope (FEI) operating at 120 kV, using a post-column energy filter (in zero-loss mode with a 20 eV energy slit width) and a CCD camera of 2048 × 2048 pixels (GIF2002, Gatan, Inc.). Tilt series consisted of up to 141 micrographs taken from -70° to 70° in 1° steps. The total dose for a series was approximately 60 e⁻/nm², and the target defocus was 4 μm under focus, putting the first zero of the CTF at $\sim (3.7 \text{ nm})^{-1}$. They were recorded at magnifications of 54,000 (3 tomograms), 38,500 (11 tomograms) and 26,000 (9 tomograms), giving pixel sizes of 0.55 nm, 0.78 nm and 1.14 nm, respectively. The intermediate magnification yielded the best tomograms (obtained from two sample preparations), providing a sufficient number of fiducial markers and particles within the field-of-view. The lower magnification appeared to decrease the quality of the clathrin spars in the tomograms. All tomograms further discussed are of the intermediate magnification.

Tomogram reconstruction

Tilt series were aligned and tomograms reconstructed using the package Bsoft (Heymann and Belnap, 2007; Heymann et al., 2008a). The tomograms were denoised by a non-linear anisotropic diffusion algorithm (Frangakis and Hegerl, 2001). Individual clathrin particles were selected and extracted. The resolution of each individual micrograph was estimated by Fourier ring correlation using a cutoff of 0.3 (FSC_{0.3}) (Cardone et al., 2005; Heymann et al., 2008a). The zero-tilt micrographs showed resolutions of 5.4 – 6.5 nm.

Modeling the clathrin lattice

Polyhedral models of the clathrin network were built into the tomographic subvolumes using the program Chimera (Pettersen et al., 2004). Symmetry was determined by visual inspection. The coordinates of the polyhedral vertices were regularized (Heymann et al., 2008b). The reference inter-spar angles were set to the canonical angle for the associated polygon (90° for a tetragon, 108° for a pentagon, 120° for a hexagon and 128.6° for a heptagon).

Modeling the vesicle membrane

The vesicles in CVs are approximately spherical. Accordingly, for each vesicle, a set of closely spaced points was generated, evenly distributed at a radius approximately equal to that of the vesicle. The model was generated and positioned relative to the tomogram by cross-correlation. The positions of points were refined by cross-correlation to a reference membrane patch. The offset of the vesicle within each CV was calculated as the difference in the geometric centers of the coat polyhedron and the vesicle model. To represent to

volume of the membrane, a shell mask with a thickness of 5 nm was generated (in a eukaryotic bilayer the P-P distance is ~4 nm (Mitra et al., 2004) and typically phospholipid bilayers are ~5 nm (Woodka et al., 2012)).

Segmenting the coated vesicle

A synthetic clathrin coat map was constructed using the polyhedron and averaged spar density from a single particle reconstruction of the 36-vertex D₆ barrel (Heymann et al., 2005). The use of this map avoids including spurious densities that might be present in a tomogram. This map was binarized by thresholding at a level that gave a good representation of the N-termini. This mask and a mask covering the region outside the polyhedral model were used to isolate the interior of the CV, excluding the coat.

The membrane model (see above) was refined within this interior mask, and a membrane mask produced as a shell with a thickness of 5 nm. The membrane mask and a mask outside the membrane model were used to isolate the vesicle lumen. The membrane mask and a mask of the vesicle lumen were used to produce a mask of the interstitial volume.

With these four masks (coat, interstitial, membrane and lumen), each region was isolated as a separate map.

Counting adaptor protein (AP) densities

The AP core-sized densities in the interstitial space of each CV were counted manually. In addition, potential APs in the interior of CBs and interstitial spaces of CVs were located as follows: The segmented map was cross-correlated with a soft-edged spherical reference density of 8 nm in diameter. Peaks in the cross-correlation map higher than 30% of the maximum correlation were identified and when two or more were closer than 8 nm, the one with the higher value was retained. Most of the densities corresponding to these peaks (> 70%) were consistent with the manually selected densities, with only small differences in assigned location (from 0 to 6 nm). Because of the greater objectivity of the automated method, it was used to give the AP numbers used in calculating their contributions to the particle masses of CBs and CVs.

Masses of components

Masses of the individual proteins were obtained from UniProtKB/Swiss-Prot (<http://www.uniprot.org>), or the RCSB/PDB (<http://www.rcsb.org>).

Clathrin bovine heavy chain: P49951, 191.6 kDa; bovine light chain A: P04973, 26.7 kDa; bovine light chain B: P04975, 25.1 kDa. The average of the light chains (25.9 kDa) was used in the calculation of the triskelion mass: $3 \times (191.6 \text{ kDa} + 25.9 \text{ kDa}) = 652.5 \text{ kDa}$.

AP-2 mass: Q0VCK5, alpha-2 103.8 kDa, P63009, beta 104.6 kDa, Q3ZC13, mu 49.7 kDa, Q17QC5, sigma 17.0 kDa. Total mass for AP-2: 275.1 kDa. The AP-2 core was estimated at 219 kDa from the residue masses in the crystal structure 1GW5 (PDB). The appendages were estimated from the crystal structures: alpha (1QTS) 26 kDa and beta (1E42) 24 kDa.

Membrane mass—Typical membrane lipids have densities of ~1 g/ml (Greenwood et al., 2006). However, the CV vesicles also contain an unspecified amount of protein. Assuming that the membrane contains about 30% protein at 1.35 g/ml, the density is estimated at ~1.1 g/ml or ~660 Da/nm³ (comparable to that estimated for synaptic vesicles - (Takamori et al., 2006)). This density was then used to estimate the vesicle membrane mass from the volume of the membrane mask (see above).

Results and Discussion

Clathrin baskets and coated vesicles

The tomograms show many coated particles, readily identifiable as such by their distinctive clathrin lattices (Figure 1A). Similar particles have been identified as coated vesicles (CVs) in cellular tomograms (Ladinsky et al., 1999; Zampighi et al., 2005). Our detailed analysis focused on 5 tomograms of ice layers that were relatively thin (80 – 160 nm, as measured directly from the tomograms) but nevertheless thick enough for the coated particles to be completely embedded. They range from ~ 66 nm to ~134 nm in diameter (average 74 nm, SD = 10 nm), as assessed from central sections. These dimensions are in agreement with earlier observations of unstained freeze-dried specimens by dark-field STEM (Steer et al., 1988) and measurements *in situ* (Hirst et al., 2008) and extend the size range covered in a previous study by cryo- ET (Cheng et al., 2007).

The smaller particles lack an internal membrane and we take them to be clathrin baskets (CBs) (Figure 1B), similar to those studied in “single particle” cryo-EM analyses of the abundant D6 basket (see Introduction). However, many of the larger ones (i.e. 80 nm in diameter) clearly contain vesicles, which are ~ 30 to 68 nm in diameter (Figure 1C). These, the true CVs, make up ~ 20% of the total population (Table 1). Similar estimates in the 25 – 30% range have been reported (Merisko et al., 1982; Steer et al., 1988). It follows that any bulk analysis of such preparations refers primarily to CBs. *In vivo* baskets are rare, most likely because the free triskelions are chaperoned (Jiang et al., 2000). Increased numbers of baskets have been observed only in cases where auxilin is overexpressed (Zhao et al., 2001) or depleted (Hirst et al., 2008), disrupting the normal ratios of CV components. CBs assemble readily *in vitro* without the participation of membranes (Keen, 1990). Since our (and other similar) preparations are made at pH 6.5, conditions that promote the polymerization of clathrin, the CBs are probably formed during the isolation procedure.

Modeling clathrin lattices

The clathrin lattices in the tomograms are well defined and were modeled as polyhedral fullerene shells. To model a given coat, a vertex site was placed at each point identified as a triskelion hub. Links were then generated between adjacent vertices, in most cases closing the polyhedron (e.g. Figures 1D,E). Subsequently, the models were refined by an automated regularization procedure (see Materials and Methods). Each model was examined to determine its symmetry and the number and types of its polygonal facets. Many different lattices (N = 54) were identified and cataloged (Table 2). Despite this overall diversity, a subset with 28 to 38 vertices accounted for the majority of these coats (196/246; ~ 80%; mostly CBs); the other geometries observed were relatively rare. Even the 54 forms observed here represent only a small fraction of the vast number of possibilities: there are 32 possible forms with 28–36 vertices (Katsura, 1983), and 5767 with 28–60 vertices (Brinkmann and Dress, 1997).

The large majority of facets in these coats are either pentagons (60.5%) or hexagons (39.0%), while some of the larger lattices also include a few tetragons (0.07%) and heptagons (0.4%) (e.g., Figure 1E). The heptagonal faces have been reported before in CVs (Cheng et al., 2007) and in flat sheets (Heuser, 1980). To our knowledge, the rarer tetragonal (square) faces have not been seen before.

As there is a one-to-one correspondence between vertices and triskelia, the number of vertices in a given model affords a measurement of the coat mass, taking the mass of an individual triskelion to be 653 kDa (Materials and Methods). The closed clathrin lattices in our data set range in mass from 18 to 56 MDa.

Vesicle size limits and size determination

35 *bona fide* CVs with closed polyhedral coats were present in our data set (Table 2). Their vesicles are generally small (minimum diameter, ~ 30 nm) and, at least approximately, spherical. The smallest CVs that we found have coats with 38 vertices and a diameter of ~80 nm, values similar to those reported by Vigers et al. (Vigers et al., 1986a), and slightly larger than the 36-vertex CV described by Cheng et al. (Cheng et al., 2007). These vesicles are similar in size to the smallest pure lipid vesicles (Haque et al., 2001; Lapinski et al., 2007; Tenchov et al., 1985). Other vesicles and the surrounding coats are larger, for instance the average coat diameter for CVs is 89 nm (SD = 10 nm) and the largest one observed in this preparation, 116 nm.

To assess the shape of a given vesicle, a spherical sheet of the appropriate radius made up of evenly spaced points was generated. This model was then used to generate a mask 5 nm thick that represents the vesicle membrane (see Materials and Methods for discussion of the assigned mean thickness). Such a model is shown in Figure 1E. The mask volume was then converted into a mass using a density of 660 Da/nm³ (Materials and Methods). Thus calculated, these vesicles range from 10 to 32 MDa in mass (excluding a large outlier at 45 MDa).

Vesicles are confined within their surrounding coats, but their sizes do not strictly follow the maximum accommodatable size (i.e., the average coat diameter minus ~28 nm) and is often smaller. Moreover, in agreement with Cheng *et al.* (Cheng et al., 2007), we find the vesicles to be offset relative to the coats. The average offset is 6 nm (SD = 3 nm) and the range covered is 2 – 13 nm. Smaller vesicles show larger offsets than bigger ones (Figure 2). Using the polyhedral models of the coats and the spherical shell models of the vesicles, we determined the closest approach between the membrane and a triskelion hub for each CV. These measurements range from 8 to 18 nm, with an average of 14 nm (SD = 3 nm). Appraisal of the models led to the conclusion that the closest a membrane can approach the hub without overlapping the N-termini of the clathrin heavy chains is about 14 nm. It therefore appears that the vesicles are close to or in contact with the clathrin N-termini on one side, leaving a crescent of interstitial space occupied by APs on the other side (Figure 3). This arrangement may reflect the geometry of the coated pit, where the APs are required to couple the coat to the membrane as invagination proceeds, while the side where the vesicle closes involves other proteins, e.g., dynamin and endophilin (Sundborger et al., 2011).

We did observe some incomplete coats (Table 1). It is therefore possible that the coat is not necessarily closed completely after scission of the vesicle. Once the support for a curved membrane is no longer required, the coat can be recycled for subsequent endocytosis.

Counting adaptor proteins (APs)

The majority of densities in the interstitial spaces of the CVs are of the right size (~8 nm diameter) and shape for the AP core (~220 kDa), and APs have been shown to be (by far) the most abundant protein component other than clathrin (Ahle and Ungewickell, 1989; Blondeau et al., 2004; Girard et al., 2005; Keen et al., 1991; Pearse and Robinson, 1984). We marked these densities with points in each CV (the green densities in Figure 3). This procedure was performed both manually and automatically. The manual method yielded on average 50 APs/CV (SD = 21), while the automated method with an appropriate threshold for selecting densities yielded 45 APs/CV (SD = 15). The automated approach therefore yielded a basically consistent result. We also searched for AP densities inside the CB interiors with the automated method, finding 34 APs/CB (SD = 9). All further references to AP counts are from the automated procedure.

The total AP mass in a given CB or CV was taken to be the number of detected points times the mass of an AP complex (275 kDa, including the appendages). The resulting numbers range from 2 to 18 MDa for CBs and from 6 to 27 MDa for CVs. The number of APs was found to correlate with the triskelion count, giving a ratio of 0.92 AP/triskelion ($SD = 0.19$, $n = 35$, $R = 0.79$) for CVs, and 1.02 AP/triskelion ($SD = 0.19$, $n = 214$, $R = 0.73$) for CBs. This result is similar to the molar ratios determined by quantitative SDS-PAGE for bulk preparations of coated vesicles (Ahle and Ungewickell, 1989; Keen et al., 1991; Pearse and Robinson, 1984) and somewhat lower than the value of ~1.5 reported from mass spectrometry (Blondeau et al., 2004; Girard et al., 2005).

Total masses of CVs and CBs

So far, we have used the cryo-tomograms and *a priori* information to estimate the mass contributions of the main parts of each CB and CV in our data set: the clathrin coat; the vesicle (only in the CV); and the APs. The sums of these parts can be compared to distributions of particle masses (Steven et al., 1983) and compositional studies (Blondeau et al., 2004) of similar preparations. The calculated masses were obtained by summing the two major parts in CBs (Figure 4A) and the three major parts in CVs (Figure 4B). In CBs, which are systematically smaller than CVs (an average diameter of 71 nm vs 89 nm), clathrin accounts on average for ~70% of the total mass. In CVs, clathrin contributes about half of the mass, although this fraction falls with increasing particle size: the remaining mass divides nearly equally between the vesicle and APs.

The masses for the CBs and CVs when combined give an expected distribution for the bulk preparation. Table 3 shows that the large proportion of CBs skews the composition such that most of the overall mass is attributed to clathrin and APs, with vesicles making only a minor overall contribution.

We cannot directly measure the total masses of individual CBs and CVs in our tomograms. In particular, the payload proteins are not accounted for. However, the distribution of two- and three-part mass sums can be compared with the distribution of particle masses determined earlier by STEM for the same type of preparation (Steven et al., 1983). Figure 5 shows the calculated distributions of masses for the CBs (gray bars) and the CVs (black bars), with a transition in the 40 – 45 MDa range. The combined distribution follows the pattern of the STEM data quite closely, reproducing the two peaks at 27 and 33 MDa associated with abundant CBs (Figure 5A, gray curve). The decrease in numbers of CVs with size is also reflected in both data sets (Figure 5B). The average mass per particle from the STEM data is 35.2 MDa, while that for the combined CB and CV populations is 35.1 MDa. The agreement between these two data sets indicates that little mass remains to be accounted for, including other accessory proteins and the CV cargoes. Separating the CBs and CVs, the average mass per particle is 31.0 MDa and 60.4 MDa, respectively. If it is assumed that the difference between the STEM and tomography data reflects only additional mass in CVs, it amounts to ~0.7 MDa per CV. Given the uncertainties in our analysis, this can vary to some extent, but not beyond a few percent of the CV mass.

Coated vesicle cargo

CV cargoes are diverse (see e.g., (McMahon and Boucrot, 2011; Pizarro-Cerda et al., 2010)). The red densities in Figure 3 illustrate the variety of shapes and levels of occupancy inside the vesicles in our tomograms. Some of the green densities in contact with the vesicle membranes may be membrane-associated or -embedded proteins. Preparations from bovine brain are likely to contain CVs with proteins typical of synaptic vesicles (Blondeau et al., 2004). The narrow size range of vesicles (30 – 50 nm, average 16 nm, $SD = 8$ nm) and their masses (average 16.4 MDa, $SD = 7.7$ MDa) in our CVs (blue shells in Figure 3A–C)

correspond to the values obtained for synaptic vesicles (Takamori et al., 2006). We therefore expect that some of the densities seen in our tomograms to represent proteins from synaptic vesicles.

It is not yet possible to identify specific cargo molecules on morphological grounds. Densities are visible protruding from the outer surface of a vesicle (formerly, the cytoplasmic surface of the cell membrane – Figure 6). These could be adaptor proteins involved in cargo selection or linking the coat to the vesicle, or parts of receptors and other membrane-embedded proteins. In many of our CVs, elongated densities extend from the outer surface of the membrane, reminiscent of the abundant synaptobrevin or the bulky V-ATPase (Figure 6B,C,D,E,K,L). Inside the vesicle lumen (formerly on the outer surface of the cell membrane), densities clearly extend beyond the membrane surface, but always with some indication of connections. The vesicle membrane appears to vary in thickness, sometimes showing distortions (Figure 6M) and large protrusions (Figure 6N,O).

Preferred curvatures of clathrin coats and vesicles

CBs are smaller, averaging only 71 nm in diameter ($SD = 6$ nm). It is likely that this size reflects the preferred (intrinsic) curvature for clathrin assembly under these conditions. This suggests that the larger coats formed around vesicles are assembled under conditions in which the growing lattice is under some stress, pushed outwards from the shape it would naturally assume and it, in turn, exerts inwards pressure on the invaginating membrane pit (soon to be vesicle). Even though the individual interactions between the triskelion legs may be weak, the coordinated effect may be sufficient for this process (Wakeham et al., 2003). In this way, the growing clathrin coat may contribute actively to the endocytic process.

However, other factors may also affect coat size. In the case of virus entry, the size must be dictated by the cargo, assuming the virus to be essentially incompressible. The bovine brain preparation studied here is likely to contain precursors to synaptic vesicles (Blondeau et al., 2004) and indeed the vesicles observed fall within the range reported for synaptic vesicles (30 – 50 nm, 10 – 30 MDa (Qu et al., 2009; Takamori et al., 2006)). As precursors to synaptic vesicles, the size range may be set in the CVs, as suggested in a recent report where reduction in the expression of the coat accessory proteins, AP180 and CALM, led to larger synaptic vesicles (Petrálie et al., 2013).

Brain CVs have relatively small payloads

CVs are large particles, being at least 80 nm in outer diameter, with coats made up of at least 38 triskelions (114 clathrin heavy chains) and total masses of ~40 MDa and more. It is striking that, as illustrated in Figure 5, almost all of the mass of a CV can be accounted for in terms of its clathrin coat, complement of APs, and the vesicle; in contrast, the cargo mass is small. We detect only 5 to 10 densities associated with the inner surface of a 30 nm vesicle. Most of them are visibly connected to the surface of the vesicle and it is likely that the others are also connected via linkers too fine to be visualized at the current resolution. Moreover, these densities are quite variable in shape, implying that a given CV takes up a variety of different cargo molecules. It may be that there are other, lower-profile, cargo proteins embedded in the lipid bilayer but still the payload cannot amount to a few percent of the particle mass. This is much less than the corresponding numbers typical of another class of carrier particle bounded by a protein coat - viral capsids. In bacteriophage HK97, for example, (Duda et al., 2009), the payload (DNA genome) accounts for ~ 60% of the nucleocapsid mass and the protein coat, ~ 40%. In the polyomavirus SV40 (Liddington et al., 1991), the payload of the chromatinized genome accounts for about 28% of the virion mass and the protein shell for the remaining 72%. In larger CVs, for instance, those that engulf entire virions in cell entry, the payload will represent a larger fraction of total particle

mass. Nevertheless, the apparent inefficiency of the trafficking processes represented by the relatively small CVs typical of brain preparations is striking.

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Abbreviations

CB	clathrin basket
CV	coated vesicle
AP	adaptor protein

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Figure 1.

(A) A field of coated particles in a 2.3 nm-thick slice through a denoised tomogram. The white arrow marks a vesicle-containing CV. The black arrow marks a CB (no vesicle). White arrowheads mark pentagonal and hexagonal facets sampled in this slice. (B & C): Serial, 3.1 nm-thick, slices through a CB (B) and a CV (C). (D & E) Ball-and-stick models of the clathrin coats of the particles in (B) and (C), also depicting the vesicle model in (C). The white arrows in panels in (C) and (E) point to the same vertex, part of a heptagonal face. The black arrow in (E) marks a tetragonal face.

Figure 2.

The offsets of vesicles relative to their coat centers are related to their size; the larger ones tend to be less eccentric and more closely follow the clathrin coat. The cartoon at top right maps the centers of a given coat (large white disk) and its enclosed vesicle (smaller white disk), connected by a double-headed arrow to indicate the offset.

Figure 3.

Cutaway renderings of CVs, including two typical ones (A: 48 triskelions, B: 58 triskelions), the smallest one (C: 38 triskelions), and the largest one (D: 86 triskelions). Each is segmented to show the clathrin coat (pink), the vesicle membrane (blue), interstitial densities (green), and cargo densities (red). In most CVs the vesicles are offset so that the lipid bilayer is close to or in contact with the clathrin N-termini (yellow arrows in A and D), while on the other side the interstitial densities, mainly APs, form a cap (crescent-shaped in cross-section) between the coat and the vesicle (white arrows in A and B).

Figure 4.

Distributions of partial masses for (A) CBs and (B) CVs. The clathrin masses (triangles) were calculated from the numbers of polyhedral vertices at 653 kDa per vertex triskelion. The AP masses (diamonds) were calculated from automated counts in the interior of each CB and the interstitial space of each CV at 275 kDa per AP. The membrane mass (gray disks) was calculated from the membrane mask volume (660 Da/nm³). The line fits and bar insets show the component fractions: (A) clathrin, 0.696 ± 0.039 ; AP, 0.304 ± 0.039 ; (B) clathrin, 0.514 ± 0.050 ; AP, 0.203 ± 0.031 ; membrane, 0.284 ± 0.052 (standard deviation).

Figure 5.

(A) Comparison of the summed masses of the clathrin, APs, and CV membranes (black bars) and of the first two components for CBs (gray bars) as calculated from the tomograms, with the distribution of total particle masses for the same kind of preparation as determined by STEM measurements (gray curve) (Steven et al., 1983). (B) An enlargement of (A) in the range of masses covered by CVs. Each bin covers 3 MDa. CVs larger than 90 MDa not shown: 2 from STEM analysis (104 and 108 MDa) and one from a tomogram (128 MDa).

Figure 6.

Gallery of central slices through 15 CVs, illustrating the wide variation in vesicle morphology and contents. The vesicle membranes exhibit local variations in thickness, resulting from embedded proteins. Some vesicles depart significantly from a spherical shape (arrows in M). Densities in vesicle lumens (arrowheads) are typically connected to the membrane, as expected for cargo proteins. Long thin densities protruding from the vesicle surface are common (arrows in B–E,G). Some vesicles have large structures attached to the membrane (arrows in N,O). Scale bar: 50 nm.

Table 1

Percentages of complete and/or vesicle-containing coated particles extracted from five tomograms (300 particles).

	Partial coat	Complete coat	Total
No vesicle	11	70	81
With vesicle	7	12	19
Total	18	82	100

Table 2

Size and symmetry of complete clathrin baskets and coated vesicles from five tomograms.

Number of vertices	Possible fullerenes	Observed symmetries	Observed forms [†]	Particle counts	Coated vesicles
20	1	-	0	-	-
24	1	-	0	-	-
26	1	-	0	-	-
28	2	T	1	61	0
30	3	C2	1	17	0
32	6	D3	1	25	0
34	6	C1,C2	4 (2)	23	0
36	15	C2,D2,D6	5 (1)	54	0
38	17	C1,C2	3 (1)	16	3
40	40	C1,C2,D2	5 (1)	11	6
42	45	C1,C2,D3,D7	5 (1)	6	3
44	89	C1,C2,D2	5 (1)	8	4
46	116	C1,C2	4 (2)	4	3
48	199	C1,C2,D2	5 (1)	5	2
50	271	C2	1	2	2
52	437	C1,D2	5 (2)	5	3
54	580	C1	1 (1)	1	1
56	924	C2	1	1	1
58	1205	C1	2 (1)	2	2
60	1812	-	0	-	-
62		C1	2 (2)	2	2
64		C1	2 (1)	2	2
86		C1	1	1	1
Total			54 (17)	246	35

[†] Forms with tetragons or heptagons in parentheses (i.e., non-fullerenes).

Symmetries: C<n>, n-fold cyclic; D<n>, n-fold dihedral; T, tetrahedral

Table 3

Total mass contributions for CVs and CBs (MDa).

	Clathrin	APs	Membrane	Total
Clathrin Baskets	4640	1985	0	6625 (75.8%)
Coated Vesicles	1112	427	575	2114 (24.2%)
Total	5752 (65.8%)	2412 (27.6%)	575 (6.6%)	8739