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Mechanical Aspects of Clathrin Cage Formation

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Summary: The principal component of the coats of endocytic vesicles involved in the receptor-mediated uptake of materials from the surfaces of eucaryotic cells is a protein known as clathrin. Clathrin normally appears as a three-legged trimer joined at a common hub (a 'triskelion') which, under appropriate conditions, co-assembles with other triskelia to form variably-sized polyhedra ('cages', or 'baskets') that contain pentagonal and hexagonal faces. The size distribution of the cages can be described by a simple energetic model which contains, as a parameter, the flexural rigidity, *EI*, of the edges (struts) of the baskets. By comparing the values of *EI* thus obtained with similar quantities determined by analyzing electron micrographs of isolated triskelia (A. J. Jin, R. Nossal, *Biophys. J.* **2000**, *78*, 1183), we infer that one role of clathrin-associated proteins known as 'assembly proteins' is to link the intertwined legs of neighboring triskelia to prevent slippage when the cages are stressed.

Keywords: clathrin; elasticity; endocytosis; self-assembly; triskelia

Introduction

Eucaryotic cells take up various constituents from their surroundings by a process known as receptor mediated endocytosis.[1,2] Central to this process is the formation of small, protein-coated vesicles that bud off from plasma membranes and carry receptors and cargo into the cells. The major constituent of the coat is clathrin, a heteropolymer consisting of a clathrin light chain (CLC) of MW ca. 25–35 kDa and a clathrin heavy chain (CHC) of MW ~190 kDa. Clathrin normally is isolated from cells in the form of trimers joined at a common hub. This complex, known as a “triskelion," has a pinwheel-like shape that enables it to assemble into cage-like, supramolecular polyhedra having pentagonal and hexagonal facets (Fig. 1). These cages (or “baskets”) are approximately 100 nm in diameter, and their morphological features resemble the seams of miniature soccer balls. In these aspects they are similar to structures found on the surfaces of native, plasma-membrane-derived, coated vesicles.

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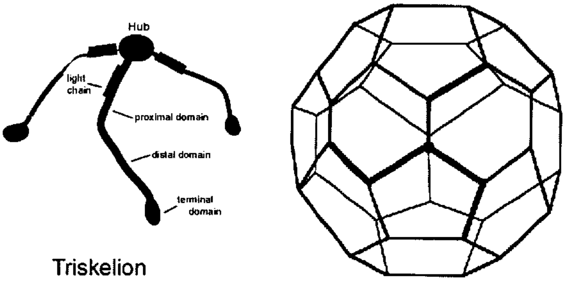
The CHCs, which constitute the legs of the triskelia, are approximately 50 nm long (and ca. 2.4 nm in cross section), but under normal physiological conditions they tend to be bent midway along their length (see Fig. 1). In accord with Euler's formula relating the topological features (viz., faces, edges, vertices) of closed polyhedra, one infers[3] that a minimum of 20 triskelions are required to form closed cages containing only pentagonal and hexagonal faces. A common small basket (the D6-barrel, having 12 pentagonal and 8 hexagonal faces[4]) contains 36 triskelia. Usually, upon studying either native coated vesicles[5] or reconstituted baskets,[5,6] one observes that even within the same preparation the cage size is somewhat variable. Quantification of baskets or coats indicates that the number of triskelia in a structure often exceeds 100,[5-7] but baskets assembled in the presence of the "AP2 adaptor complex" are smaller and more uniform than baskets assembled only from clathrin.[6] Among the large group of proteins that are known to associate with clathrin,[8] several, including AP2 adaptors, have been designated as "assembly proteins" (APs) because they facilitate basket formation under conditions inimical to the formation of baskets from clathrin alone. The average size of the clathrin coats associated with vesicles harvested from different sources varies with tissue type,[5] perhaps reflecting differences in the physical properties of the enclosed membrane, as well as differences in associated molecules. Also the type and size of the cargo can affect the rate of formation and dimensions of the budded vesicles.[9]



Triskelion

Basket

Figure 1. Cartoon indicating the shape of an isolated clathrin triskelion and a polyhedral "basket" formed by the interaction of two proximal and two distal segments of adjoining triskelia. Cryoelectron microscopy[4] shows that the legs interwind and that the terminal domains lie within the interior of the basket

Protein-coated tubovesicular structures are involved, also, in other intracellular trafficking activities.[10] In particular, COPI and COPII coats mediate the formation of transport vesicles involved in transfer of materials between the Golgi apparatus and the endoplasmic reticulum (ER). However, the architectures of native COPI and COPII[11] coats are known at much lower resolution than that of the clathrin coat. Moreover, although cage-like particles recently have been assembled from solutions of COPII proteins,[12] their structure is only poorly defined. Hence, clathrin coats, because of their distinct morphology,[13] offer unique advantages if one is interested in quantitative questions relating to mechanisms by which transport vesicles arise. Also, in contrast to other large multi-unit, but linear, polymers which are potentially of indefinite length, clathrin cages, like virus capsids[14] and single-walled tubulin rings (formed in association with various small peptides[15]), are closed polymers of finite size. However, despite the fact that the structures are closed, they can (at least, in the case of clathrin cages) vary in size. These structures therefore may be of intrinsic interest to polymer physicists, since the principles by which they are put together may differ somewhat from those for assembling linear polymers.

Previous Studies

We previously considered various mathematical and physical questions relating to clathrin cage assembly, including how topological constraints imposed by triskelion geometry might mediate the generation of a vesicle coat from a pre-existing membrane-bound clathrin patch.[3] We also inferred the strength of clathrin lattices by analyzing the shape fluctuations of clathrin triskelia prepared for electron microscopy by glycerol nebularization,[16] comparing the results with published values of the rigidities of cell membranes.[17] Interestingly, we found that the flexural rigidity, EI, of the plasma membrane is similar in magnitude to that of a clathrin coat if one assumes that the intertwined legs are strongly fixed to each other when they form a strut between two triskelial hubs (the latter being the vertices of the polyhedra). This is an important result, as it implies that although the coat can aid in stabilizing a basket, some other mechanism may be required to initiate CCV formation. (Recent work has shown that certain clathrin-associated proteins may bind to particular lipid constituents (phosphoinositides), thereby changing the curvature of the membrane.[18,19]) However, the struts would be much weaker were the clathrin legs to slip with respect to each other, and the mechanical role of the lattice would be significantly diminished. What might link the legs together? A good candidate is the adaptor complex, AP2,[20] or similar "assembly protein" (e.g., AP180 [21]) which, as mentioned above, binds to clathrin and facilitates the formation of closed cages. Although to investigate the role of such factors one ideally would like to make direct mechanical measurements on sparse biomimetic systems, only limited progress has been made in reconstituting a lipid/protein system that binds clathrin[22] and procedures for making quantitative measurements of mechanical properties of such systems have not yet been worked out. (Recently, though, the mechanical properties of intact, native CCVs have been determined.[23])

Fortunately, the rigidities of clathrin structures can be estimated by making use of data on size distributions of reassembled clathrin cages.[7] We do so by relating the basket size distributions to an expression for the net free energy change occuring when baskets form, the latter being composed of a term accounting for the energy required to distort triskelia as they fit into the baskets and another that represents the net favorable energy due to interactions between interwound neighbors. The resulting free energy expression contains three parameters – a rigidity coefficient, intrinsic triskelial shape parameter, and a term signifying the net inter-leg interaction energy – which can be estimated by fitting the theory to experimental data. This model rationalizes the limited range and dispersion noted in basket sizes. Additionally, inferred parameter values indicate that the net (favorable) free energy change resulting when baskets form in the presence of APs is greater than without APs. In both cases, results show that basket assembly involves lattice rearrangements driven solely by thermal mechanisms,[7] in accord with both in vivo and in vitro experimental observations. One also can obtain estimates of the effective rigidity modulus associated with distorting the basket struts. In this note we use a variant of our earlier model in which, now, the distortion energy is expressed in terms of leg curvature, and thereby obtain an independent estimate of the flexural rigidity of a basket strut, EI_{strut}. By comparing results based on distributions of baskets formed with and without APs, we show that an important effect of binding APs and other assembly proteins to the lattice indeed is to prevent the legs in a strut from slipping with respect to each other when a basket is stressed.

Model for Free Energy Change During Basket Assembly

We write the free energy change, G_N, that occurs during formation of a basket composed of N triskelia as[7]

$$G_N = G_N^{dist} + G_N^{stab} = N(g_N^{dist} + g_N^{stab}), \quad (1)$$

where the distortion energy per triskelion, g_N^{dist}, here is written as

$$g_N^{dist} = h\,(1/N^{1/2} - 1/n^{1/2})^2 \quad (2)$$

and the stabilization energy per triskeion, g_N^{stab}, is given, to first order, by

$$g_N^{stab} = b'\,. \quad (3)$$

In these equations, the coefficient h is related to the rigidity of the triskelion legs, n is a marker of the intrinsic shape of the triskelion, and the term b', which contains both enthalpic and entropic components, represents the net average energy of clathrin interactions. Although typical clathrin baskets are polyhedra containing 12 pentagonal faces and a variable number of hexagonal facets,[5] we implicitly assume that essential properties can be discerned if the baskets are approximated as spherical shells in which all triskelia experience an equivalent environment. Hence (cf. Eq. (1)),

$$G_N = hN(1/N^{1/2} - 1/n^{1/2})^2 + b'N\,. \quad (4)$$

The first term on the r.h.s. of Eq. (4), i.e., G_N^{dist} (cf. Eq. (2)), is similar to an expression often used to model membrane mechanics.[24] It can be derived by assuming[17] that the bending energy of a strut joining two triskelial hubs is $\frac{1}{2}\int_0^{L/2} EI(s)\,(C(s) - C_0(s))^2\,ds$, where $(C(s) - C_0(s))$ is the deviation of the curvature, $C(s)$, of the strut from its "natural" unperturbed value, $C_0(s)$. L approximates the length of a triskelion leg, and $EI(s)$ is a position-dependent flexural rigidity coefficient. If we assume a fixed curvature along the entire edge, viz., $C = 1/R$, and take $EI(s) \approx$ const. $= EI_{strut}$,[17] we obtain, for the energy required to distort each edge,

$$G^{dist}(strut) = (L/2)\cdot(EI_{strut}/2)\cdot(1/R - 1/R_0)^2\,, \quad (5)$$

where $1/R_0$ is a measure of the natural curvature of the triskelions. Because G_N^{dist} is the distortion energy per strut multiplied by the total number of struts, Eq. (5) leads to

$$G_N^{dist} = (3/8)\cdot N\cdot L\cdot EI_{strut}\cdot(1/R - 1/R_0)^2 , \qquad (6)$$

since the number of edges equals 3/2 the number of triskelions. A similar expression has been used to describe the mechanics of a thin shell of actin filaments located adjacent to the inner surface of a spherical bilayer vesicle.[25]

The effective diameter, d, of a basket can be related to its size, N, as $N \approx 0.031d^{7/4}$ where $d=2R$ is given in nm.[7] The size distribution of baskets formed in the presence of APs has a peak at $d^* \approx 78$ nm, and for baskets formed from clathrin alone, at $d^* \approx 99$ nm.[6] Thus, from the relationship $N \approx 0.031d^{7/4}$ we find[7] $N^* \approx 64$ (with APs) and $N^* \approx 96$ (without APs) so, in the size range of interest, we can use the relationship $N \approx 0.01,\ d^2 \approx 0.04\ R^2$. It immediately follows (c.f. Eqs.(4) and (6)) that h is related to the flexural rigidity by $h \approx 0.015\cdot L\cdot EI_{strut}\ (nm)^{-2}$. Finally, although the length of a triskelion leg is approximately 50 nm,[16] the effective length of a strut within a clathrin basket is close to 18.5 nm rather than 25 nm,[26] perhaps reflecting the fact that the legs are intertwined. If we use the value $L_{equiv} = 37$ nm, we then find,

$$EI_{strut} \approx 1.8\ h \cdot nm \qquad (7)$$

where h has the units of k_BT.

Estimates of Parameter Values

We fit our theory to the relative numbers, P_N, of baskets of size N. Assuming that basket formation occurs by a quasi-thermodynamic mechanism (at least, up to a critical point in their assembly[7]), it follows that to first order the size distribution may be written as

$$P_N \sim \exp(-\beta G_N) \sim \exp(-\beta[(1/N^{1/2} - 1/n^{1/2})^2 - b']N), \qquad (8)$$

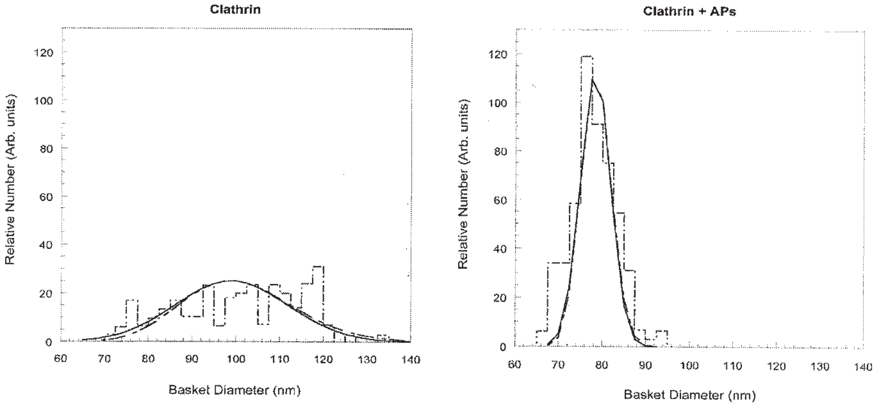
where $\beta = (k_BT)^{-1}$. Simple closed form expressions for the maximal value, N^*, and width, $W_{1/2}$, of the basket size distribution given by Eq. (8) may be derived which are similar to analogous expressions derived previously for a related model.[7] Here, $W_{1/2} \equiv N_+ - N_-$, where $N_{+/-}$ are the values of N for which $P = ½\ P_{N^*}$. We find

$$N^* = n(1 - b'n/h)^{-2}, \qquad (9)$$

and

$$W_{1/2} = 4\,N^*\,((\beta h)^{-1}\,\Psi\,\ln 2)^{1/2} \qquad (10)$$

where $\Psi \equiv (1 - b'n/h) = (n/N^*)^{1/2}$. These serve as two of three relations needed to determine the parameter group $\{h, n, b'\}$.


Clathrin

Clathrin + APs

Figure 2. Size distributions of reconstituted baskets formed in the absence (left) and presence (right) of assembly proteins. The histograms are data determined by electron microscopy of basket suspensions,[6] while the solid curves represent size distributions calculated according to Eq. (8) when the parameters are determined according to Eqs. (9) and (10) and an assumption that baskets do not form if the net free energy change upon polymerization is greater than zero (see text). The dotted lines show fits obtained by using a similar model in which the distortion energy is related to the solid angles subtended by triskelia when they are incorporated into a basket.[7]

As before,[7] the third relationship can be obtained by making an assumption about the value of the free energy, G_0, for which $P_N = 0$. Here we assume that $G_0 = 0$, which implies that baskets are unstable and come apart if the net free energy of assembly is positive. (The inferred parameters, particularly h, are relatively insensitive to the choice of G_0.) We thereby find the values $\{b' = 0.24\ k_BT,\ n = 58.7,\ h^{clath+APs} = 335\ k_BT\}$ for baskets assembled with APs, and $\{b' = 0.046\ k_BT,\ n = 77.7,\ h^{clath} = 35.4\ k_BT\}$ for baskets assembled from clathrin alone. The main point here is that the mechanical rigidity of baskets assembled with APs is an order of magnitude greater than when assembled from clathrin alone: from Eq. (7) and the values of h^{clath} and $h^{clath+APs}$, we find $EI_{clath} \approx 63\ k_BT{\cdot}nm$ and $EI_{clath+APs} \approx 600\ k_BT{\cdot}nm$.

Discussion

We previously investigated the mechanical properties of clathrin legs by analyzing electron microscopy images[16] of triskelia prepared by glycerol nebularization and rotary shadowing. The variable triskelial shapes noted in the images were presumed to mimic the fluctuating shapes of triskelia in solution, thereby allowing us to use equilibrium statistical mechanics to estimate the leg rigidity.[17] Although the analysis depended upon a number of significant assumptions, it was argued that the value determined for EI_{leg}, viz., $EI_{leg} \approx 35$ $k_BT \cdot nm$, is at least qualitatively informative. We also estimated EI_{strut}, the flexural rigidity of a strut lying between neighboring triskelial hubs in the lattice. To do so, we assumed $EI_{strut} \approx E \cdot I$, where E is an effective Young's modulus for the composite structure made up of two proximal and two distal leg segments and I is the geometrical moment of inertia. We calculated I for several plausible cross-sectional shapes and set $EI_{strut} \approx 16 \cdot EI_{leg}$, which was close to the average and is equivalent to assuming the mass of the strut to be uniformly distributed within a cross-sectional area whose radius is twice that of an individual leg. (The moment of inertia for a structure with circular cross-section is $I = \pi R^4/4$.) It then follows that $EI_{strut} \approx 560$ $k_BT \cdot nm$. However, we stress that this value holds only if the legs are strongly attached; the effective value of EI_{strut} will be lower if the legs move with respect each other when the lattice is stressed.

In comparison, from our above analysis of basket reconstitution data, we infer that in the absence of APs the rigidity of a strut, EI_{clath}, is approximately 63 $k_BT \cdot nm$. (EI_{clath} in fact is somewhat higher if, as suggested,[6] the supposedly AP-free clathrin sample was slightly contaminated with AP-containing baskets; upon removing their contributions to the data, the width of the distribution would be narrower, yielding higher rigidity.[7]) This value is greater than, but of the same magnitude as, that for an isolated clathrin filament ($EI_{leg} \approx 35$ $k_BT \cdot nm$), suggesting that in the absence of APs the component filaments of the legs slide with respect to each other. In contrast, the value of $EI_{clath+APs}$ (≈ 600 $k_BT \cdot nm$), which is in remarkably good accord with the value inferred from the shape fluctuations ($EI_{strut} \approx$ 560 $k_BT \cdot nm$), is consistent with the notion that the filaments are bound together by the APs. The increase in the rigidity of lattice struts agrees with our observation that APs strengthen interactions between the triskelion legs, as manifest by an increase in b'.

The details of our energetic model may not be as important as the general view that basket formation is driven by attractive forces between triskelia, counterbalanced by unfavorable distortion energies. Our data-fitting procedure probably is consonant with several variations of this overall model. One might, for example, also include torsional stress or extensions (stretching) of the triskelion legs. Yet, it is hard to envision how such elements would affect the main conclusion, namely that a significant role for "assembly proteins" is to fasten the legs together to prevent slippage when a basket is subject to mechanical stress.

[1] T. Kirchhausen, *Annu. Rev. Biochem.* **2000**, *69*, 699.
[2] F. M. Brodsky, C-Y.Chen, C. Knuehl, M. C. Towler, D. E. Wakeham, *Annu. Rev. Dev. Biol.* **2001**, *17*, 517.
[3] A. J. Jin, R. Nossal, *Biophys. J.* **1993**, *65*, 1523.
[4] C. J. Smith, N. Grigorieff, B. M. F. Pearse, *EMBO J.* **1998**, *17*, 4943.
[5] J. Heuser, T. Kirchhausen, *J. Ultrastruct. Res.* **1985**, *92*, 1.
[6] S. Zaremba, J. H. Keen, *J. Cell Biol.* **1983**, *97*, 1339.
[7] R. Nossal, *Traffic* **2001**, *2*, 138.
[8] E. M. Lafer, *Traffic* **2002**, *3*, 513.
[9] M. Ehrlich, W. Boll, A. Van Oijen, R. Hariharan, K. Chandran, M. L. Nibert, T. Kirchhausen, *Cell* **2004**, *118*, 591.
[10] J. S. Bonifacino, B. S. Glick, *Cell* **2004**, *116*, 153.
[11] K. Matsuoka, R. Schekman, L. Orci, J. E. Heuser, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 13705.
[12] B. Antony, P. Gounon, R. Schekman, L. Orci, *EMBO Reports* **2003**, *4*, 419.
[13] A. Fotin, Y. Cheng, P. Sliz, N. Grigorieff, S. C. Harrison, T. Kirchhausen, T. Walz, *Nature* **2004**, *432*, 573.
[14] A. Zlotnick, *Virology* **2002**, *315*, 269.
[15] H. Boukari, R. Nossal, D. L. Sackett, P. Schuck, *Phys. Rev. Lett.* **2004**, *93*, 098106.
[16] E. Kocsis, B. L. Trus, C. J. Steer, M. E. Bisher, A. C. Steven, *J. Struct. Biol.* **1991**, *107*, 6.
[17] A. J. Jin, R. Nossal, *Biophys. J.* **2000**, *78*, 1183.
[18] M. G. J. Ford, I. G. Mills, B. J. Peter, Y. Vallis, G. J. K. Praefcke, P. R. Evans, H. T. McMahon, *Nature* **2002**, *419*, 361.
[19] B. Habermann, *EMBO Rep.* **2004**, *5*, 250.
[20] J. Keen, *J. Cell Biol.* **1991**, *105*, 1989.
[21] W. Ye, E. M. Lafer, *J. Neurosci. Res.* **1995**, *41*, 15.
[22] M. G. Ford, et al., *Science* **2001**, *291*, 1051.
[23] A. J. Jin, K. Prasad, P. D. Smith, E. M. Lafer, R. Nossal, (in preparation).
[24] W. Helfrich, *Z. Naturforsch.* **1973**, *28c*, 693.
[25] W. Häckl, W. Bärmann, E. Sackmann, *Phys. Rev. Lett.* **1998**, *80*, 1787.
[26] G. P. A. Vigers, R. A. Crowther, B. M. F. Pearse, *EMBO J.* **1986**, *5*, 2079.