Mathematical modelling of the formation of coated vesicles

A theoretical geometrical approach

Michael P. LISANTI and Saul PUSZKIN*

Laboratory of Molecular Pathology, Department of Pathology, Mount Sinai School of Medicine of the City University of New York, 1 Gustave L. Levy Place, New York, NY 10029, U.S.A.

(Received 6 February 1985/25 April 1985; accepted 14 May 1985)

The mechanism by which flat hexagonal lattices of clathrin trimers transform into pentagonal/hexagonal spheres remains a mystery. In light of the geometrical nature of this process we have pursued a mathematical approach to the question. Through the geometrical analysis of flat hexagonal lattices we have discovered three possible forms of transformation to introduce curvature into the centre of the lattice: hub–centre transformation; hub–edge transformation; fringe transformation. Hub–edge and fringe transformations are used first to close the lattice while introducing localized curvature at the edges of the lattice. Hub–centre transformation is used after closure to relax the severely localized curvature generated during closure. This scheme not only maximizes the size of the coated vesicle generated, but also minimizes the number of transformations, thus minimizing the energy expended.

Coated vesicles, which function in the uptake of a variety of ligands in a host of cell types, possess size ranges specific to those cell types in which they are observed (Crowther *et al.*, 1976; Pearse, 1976). Regardless of the tissue from which they are isolated, all coated vesicles are surrounded by an array of regularly distributed pentagons and hexagons (Crowther *et al.*, 1976). This coat contains essentially two protein components: clathrin heavy chains (M_r 180000) and clathrin light chains [M_r 35000 (Kirchhausen & Harrison, 1981; Ungewickell & Branton, 1981; Lisanti *et al.*, 1982*a,b,c*)]. Heavy and light chains are associated in a 1:1 molar ratio, with three heavy-chain/light-chain complexes making up the assembly unit of the lattice, namely the clathrin trimer or triskelion (Fig. 1*a*). These triskelions are anchored on the outer surface of the vesicle membrane by 100000- M_r protein components (Unanue *et al.*, 1981).

The recent information explosion with regard to the structure and function of coated vesicles has led to the formulation of two theoretical models, each of which attempts to explain the mechanism behind the formation of coated vesicles (Katsura, 1983; McKinley, 1983). However, both models are

inherently incomplete, since neither is based explicitly on experimental evidence.

In this paper we present a model for the formation of coated vesicles that is based essentially on eight experimental observations. [A preliminary account of our work can be found elsewhere (Lisanti *et al.*, 1984).]

Theory

Key experimental observations

Essentially eight experimental clues are necessary for deducing a mechanism for the formation of coated vesicles, four of which are biochemical and four of which are morphological.

The biochemical observations are as follows.

(i) All coated vesicles are constructed on the same geometrical principle, i.e. they consist of 12 pentagons and a variable number of hexagons (Crowther *et al.*, 1976).

(ii) Clathrin trimers pack side to side in a cross-over manner to form polygonal networks [Figs. 1*c* and 1*d* (Crowther & Pearse, 1981)]. The distal portions of the trimer's bent legs may be removed by trypsin treatment without impairing their assembly capacity, indicating that the clathrin–clathrin binding site is located in the proximal portions of the legs (Schmid *et al.*, 1982). For simplicity, we have represented the clathrin trimers in their truncated (Y-shaped) form (Fig. 1*b*).

Abbreviation used: CLT, clathrin lattice transformation.

* To whom all correspondence should be addressed.

Fig. 1. Assembly units of clathrin cages and their packing into lattices

(a) The clathrin trimer or triskelion. (b) The truncated clathrin triskelion after limited proteolysis. These trimers are still assembly-competent, indicating that the clathrin-clathrin binding site is present on the inner, or proximal, portion of its bent legs. Clathrin trimers pack side to side in a cross-over manner. The packings of a tri-trimer (c) and a penta-trimer (d) are illustrated. The tri-trimer configuration and penta-trimer configuration depicted are important in defining physical transformation of the clathrin lattice.

(iii) Purified triskelions spontaneously form empty coats (devoid of vesicle membranes), at neutral or slightly acidic pH, with an apparent negative enthalpy of reaction (Schook *et al.*, 1979; Keen *et al.*, 1979; VanJaarsveld *et al.*, 1981).

(iv) An uncoating ATPase, containing one molecule of bound ATP per molecule, hydrolyses the ATP on contact with polymerized clathrin (the disassembled form of clathrin has no effect) and releases the trimer from the lattice. The ATPase itself is bound to the centre of the triskelion, thereby inhibiting subsequent polymerization. One 70000- M_r ATPase molecule binds per 215000- M_r leg of the clathrin triskelion, consuming one ATP molecule per leg released. Assembly is thus spontaneous, whereas disassembly requires energy (Schlossman *et al.*, 1984; Braell *et al.*, 1984).

The following morphological clues used in the construction of our model are contained in the freeze-etched platinum replicas of the underside of the fibroblast membrane obtained by Heuser (1980).

(i) Flat, purely hexagonal, lattices of clathrin are transformed into the resulting pentagonal/hexagonal coat structure.

(ii) Pentagons, hexagons and septagons are observed during the intermediate stages of the formation of coated vesicles, whereas septagons appear to be absent from the final coat structure. (The possibility that other polygons are involved as intermediates cannot be completely excluded; they may be so energetically unstable that their life-times are too short for them to be observed.)

(iii) Transformation is restricted to the edges of the flat hexagonal lattices, leaving the centre

Fig. 2. Three distinct polygonal configurations exist in clathrin lattices

Configuration (a) consists of four polygons (A, B, C and D) connected by two vertices; configuration (b) consists of three polygons (A, B and C) connected by two vertices; configuration (c) consists only of a single polygon (A) with at least one trimer containing a single unoccupied binding site. The detailed arrangement of trimers forming the two vertices in configurations (a) and (b) are shown in Fig. 1(d), and the arrangement of the trimer in configuration (c) containing a single unoccupied binding site is depicted in Fig. 1(c).

almost purely hexagonal, thus favouring closure of the lattice over curvature.

(iv) A large decrease in area of the lattice is observed during the transformation process; this decrease in area can be calculated explicitly for the fibroblast.

The percentage change in area is given by:

$$100(4\pi R_c^2 - N_6 A_6)/N_6 A_6 \quad (1)$$

where $N_6 A_6$ and $4\pi R_c^2$ represent the area of the lattice and cage respectively. The area of a regular n -gon is given by:

$$A_n = \frac{1}{2}nl^2 \cdot \cot(180/n) \quad (2)$$

where l is the length of each side.

For polygons constructed of clathrin trimers $l = 18.6\text{nm}$ (Crowther & Pearse, 1981) and $A_6 = 898.8\text{nm}^2$; for the fibroblast $N_6 = 61$ and $R_c = 50\text{nm}$ (Heuser, 1980). Thus the percentage change in area is -42.7% .

Clathrin lattice transformation (CLT)

Formation of coated vesicles begins with flat hexagonal lattices (Heuser, 1980). Through an analysis of the geometry of such lattices we find that the packing scheme (Crowther & Pearse, 1981) gives rise to essentially three different configurations of polygons: (a) four polygons connected by two vertices found in the centre of the lattice; (b) three polygons connected by two vertices found on the edge of the lattice; (c) one polygon with a trimer containing an unoccupied binding site found on the edge of the lattice. These three configurations are shown in Fig. 2, with the polygons involved denoted A, B, C and D.

Isolating these three forms of polygon organization from the lattice enhances visualization of possible transformations (Fig. 3). Inasmuch as a large decrease in area of the clathrin lattice is observed during closure into cages, and since an uncoating ATPase exists, it is reasonable to assume that the transformation process involves removal of trimers from the lattice. Thus, to visualize a transformation mechanism, trimers must be removed from vertices of the aforementioned polygonal configurations. Newly generated binding sites resulting from the removal of trimers thus would associate with other newly generated binding sites, since assembly is spontaneous (only disassembly requires energy), thus closing polygons opened by the removal of trimers.

Removing a single trimer from configuration (a), where polygons A, B and C are joined, results in three unoccupied binding sites in the lattice (Fig. 3a). Two of these binding sites may overlap and close the ring, forming polygon E, leaving a single unoccupied binding site. Inasmuch as this trimer is partially dissociated, it is reasonable to remove it completely. This generates two additional unoccupied binding sites, which may overlap and close the ring, forming polygons F and G. Thus four polygons (A, B, C and D) have been transformed into three polygons (E, F and G) simply by removing two trimers from the lattice.

A similar transformation scheme was devised for configuration (b), where two trimers were removed from the lattice, transforming three polygons (A, B and C) into two (E and F) (Fig. 3b).

Only a single trimer need be removed to transform configuration (c), leaving two unoccupied binding sites. These binding sites may close the ring, the number of polygons remaining constant (A becomes E) (Fig. 3c).

Since configurations (a) and (b) both require the

removal of two trimers for transformation and the removal of these trimers occurs at the vertex or centre of these configurations, these two transformations have been termed hub transformations (H). Transformation of configuration (a) differs from transformation of configuration (b) in that (a) is present only in the centre of the lattice and configuration (b) is present only at the edge. Subscripts may be attached to positionally distinguish these two forms of transformation: hub-centre (H_c) and hub-edge (H_e) transformations.

Configuration (c) is present only at the edge, or fringe, of the lattice, and is distinct from configurations (a) and (b) in terms of transformation because it requires only the removal of a single trimer. As a result, this transformation was grouped separately and positionally termed fringe transformation (F). Co-ordinate notation was used to readily describe each of these transformations, noting number of sides of each polygon before and after transformation. Configuration (a) may be represented by $N_A, (N_B, N_C), N_D$, configuration (b) by $N_A, (N_B, N_C)$, and configuration (c) by N_A .

Thus H_c , H_e and F transformations may be described similarly with the use of this notation:

$$\begin{array}{lll} H_c & N_A, (N_B, N_C), N_D \rightarrow N_E, N_F, N_G \\ H_e & N_A, (N_B, N_C) \rightarrow N_E, N_F \\ F & N_A \rightarrow N_E \end{array} \quad (3)$$

where an arrow is used to denote transformation.

When A, B, C and D are all hexagons, H_c , H_e and F are:

$$\begin{array}{lll} H_c & 6, (6, 6), 6 \rightarrow 5, 8, 5 \\ H_e & 6, (6, 6) \rightarrow 5, 8 \\ F & 6 \rightarrow 5 \end{array}$$

Thus the generalized functional relationship is: $N_E = N_A - 1$, $N_F = N_B + N_C - 4$ and $N_G = N_D - 1$.

Fig. 3. Transformation of configurations (a), (b) and (c)

All three configurations were isolated from the lattice to investigate their transformation. By the removal of the two trimers forming the vertices that connect the polygons in configurations (a) and (b), these configurations can be transformed. Only a single trimer need be removed to transform configuration (c).

Now all three transformations may be represented simply in terms of the polygons initially present (A, B, C and D):

$$\begin{array}{ll} H_c & N_A, (N_B, N_C), N_D \rightarrow N_A - 1, N_B + N_C - 4, N_D - 1 \\ H_e & N_A, (N_B, N_C) \rightarrow N_A - 1, N_B + N_C - 4 \\ F & N_A \rightarrow N_A - 1 \end{array}$$

(4)

These three transformations are defined generally in Fig. 4, where circles are used to represent *n*-gons. Mathematically, therefore, all three transformations may be seen as part of the same transformation, where H_c is unrestricted, H_e excludes polygon D, and F excludes polygons B, C and D. Thus all three transformations, H_c , H_e and F, are part of the same CLT function:

$$N_A, (N_B, N_C), N_D \rightarrow N_E, N_F, N_G$$

(5)

Restriction of the CLT function to pertinent values

Having deduced a function to represent the geometrical transformation of clathrin lattices, this function now may be restricted to the numerical values observed experimentally. Since only pentagons, hexagons and septagons may be found during the transformation of flat hexagonal lattices into closed cages (Heuser, 1980), we restricted the range and domain of CLT function to the values $N = 5, 6$ and 7 (see Appendix to Lisanti *et al.*, 1984).

Since H_c transformation is the geometrically unrestricted form of the CLT function (containing A, B, C and D), the H_c transformations are simply:

$$N_A, (N_B, N_C), N_D \rightarrow N_E, N_F, N_G$$

6, (5, 5), 6	5, 6, 5
6, (5, 6), 6	5, 7, 5
6, (5, 5), 7	5, 6, 6
6, (5, 6), 7	5, 7, 6
7, (5, 5), 7	6, 6, 6
7, (5, 6), 7	6, 7, 6

By geometrically restricting the CLT function the allowed H_e and F transformations similarly may be found. Since polygon D is absent in the H_e transformation, the allowed transformations are found simply by ignoring N_D and the resulting N_G . The H_e transformations are thus:

$$N_A, (N_B, N_C) \rightarrow N_E, N_F$$

6, (5, 5)	5, 6
6, (5, 6)	5, 7
7, (5, 5)	6, 6
7, (5, 6)	6, 7

Similarly, F transformations can be found by ignoring N_B , N_C and N_D and the resulting N_F and N_G . Thus only two F transformations are possible:

$$N_A \rightarrow N_E$$

6	5
7	6

So, by restricting the domain and range of the CLT function to values dictated by electron-microscopic evidence ($N = 5, 6$ and 7), 12 transformations have

been found that may be employed in transforming flat hexagonal lattices into closed spherical cages. At this point, two key questions arise. Is it necessary to use all or only a few of these transformations to generate cages? Does the order in which these transformations are used affect the final geometry of the cage? Again, the answers lie in experimental observation, and this is taken up in the next subsection.

Selection of CLT involved solely in closure of lattices

Experimentally, transformation of flat hexagonal lattices appears to be restricted to the lattice's fringe or edge, leaving the centre almost purely hexagonal (Heuser, 1980). We have distinguished three different forms of transformation on the basis of the number of trimers removed and position in the lattice. It becomes clear that H_c must be put aside for now, since transformation in the centre of the lattice was not initially observed. Thus six of the 12 possible transformations that

Fig. 4. Hub-centre (H_c), hub-edge (H_e) and fringe (F) transformations

The transformation of configurations (a), (b) and (c) in Fig. 3 is generalized and illustrated here, with circles being used to represent *n*-gons. The number of sides of each polygon is denoted by using a form of co-ordinate notation.

can be used in the process of lattice closure have been eliminated.

A whole series of interdependencies among H_e and F transformation may be discovered, remembering that transformation starts with a purely hexagonal lattice (Fig. 5). Notice that, in this scheme of interdependency, no septagons enter the centre of the lattice, thus preventing the possible use of two H_e transformations [7, (5,5) $\rightarrow$ 6, 6 or 7, (5, 6) $\rightarrow$ 6, 7]. This leaves only four transformations necessary for closure of flat hexagonal lattices:

H_e

F

$6, (5, 5) \rightarrow 5, 6$
 $6, (5, 5) \rightarrow 5, 7$
 $6 \rightarrow 5$
 $7 \rightarrow 6$

Now that the transformations needed for closure have been isolated, attention must be given to generating flat hexagonal lattices that may be transformed into closed cages by using these four transformations.

Regular lattices and their transformation into cages: closure and relaxation of curvature

Experimental observation may be used to construct lattices of hexagons that are built on the same principles employed by the cell. By observing Heuser's electron micrographs of flat lattices, it becomes apparent that the lattices consist of a core surrounded by concentric layers or rings of hexagons (Heuser, 1980). The central hexagon may act as a core, or nucleation site for the generation of the surrounding rings of hexagons. We begin our discussion with lattices that contain a single hexagon as their core, since these represent the simplest lattices that can be generated.

To attain a more quantitative picture of these flat hexagonal lattices, a single parameter was defined, characteristic of each lattice, that can be used to derive other useful characteristics of the lattice, namely the number of hexagons on an edge ($N_{6,e}$) (Fig. 6a).

The $N_{6,e}$ is the set of natural numbers [$N_{6,e} = 1, 2, 3, \dots$].

The next parameter that we define is the number of hexagons across the central row ($N_{6,c}$). This is given by the relation:

$$N_{6,c} = 2N_{6,e} - 1 \tag{6}$$

When $N_{6,e}$ is used to evaluate $N_{6,c}$, $N_{6,c}$ is found to be the set of all odd natural numbers.

The numbers of hexagons around the lattice at its periphery ($N_{6,p}$) is similarly defined by using the $N_{6,e}$:

$$N_{6,p} = (\sum N_{6,e}) - 6 \tag{7}$$

Finally, the total number of hexagons present in

Fig. 5. Interdependency among H_e and F transformations. If H_e transformations are ignored, certain H_e transformations become impossible, i.e. 7, (5, 6) $\rightarrow$ 6, 7 and 7, (5, 5) $\rightarrow$ 6, 6. A scheme of interdependency is depicted that indicates that only two H_e transformations [6, (5, 5) $\rightarrow$ 5, 6 and 6, (5, 6) $\rightarrow$ 5, 7] and two F transformations [6 $\rightarrow$ 5 and 7 $\rightarrow$ 6] are possible in the absence of H_e transformations,

the entire lattice (N_6) was numerically defined as:

$$N_6 = 1 + \sum_{K=0}^{N_{6,e}-1} 6K \tag{8}$$

Thus, collecting all four parameters for each individual lattice yields the following:

$N_{6,e}$	$N_{6,c}$	$N_{6,p}$	N_6
1	1	0	1
2	3	6	7
3	5	12	19
4	7	18	37
5	9	24	61 etc.

We see that the $N_{6,p}$ is simply the result of the concentric addition of increasing integral values of

Fig. 6. Construction of regular lattices for transformation (a) A hexagonal lattice represented by an enlarged hexagon is shown, and the parameters used to enumerate the properties of regular lattices are defined. These are the number of hexagons in the central row ($N_{6,c}$), the number of hexagons on an edge ($N_{6,e}$) and the number of hexagons around the perimeter of the lattice ($N_{6,p}$). (b) Regular lattices were constructed by building concentric rings of hexagons around a central core hexagon. The number of hexagons in each additional ring increases by 6, as depicted in this bull's-eye diagram of a regular lattice. The number of hexagons in the final ring is defined as $N_{6,p}$.

six hexagons (Fig. 6b). The N_6 is just a single hexagon plus the sum of increasing integral values of 6, a function resulting from the concentric addition of hexagons at the periphery of smaller lattices. These lattices (excluding $N_{6,e} = 1$ and 2) are arranged by increasing size in Fig. 7.

Having now described the geometrical periodicity of these lattices numerically, we will attempt to transform them into closed cages. We must exclude the lattice containing $N_{6,e} = 2$, since it has only seven polygons ($N_6 = 7$) and a cage must contain 12 pentagons. Thus we will start the transformation process by using the lattice with $N_{6,e} = 3$. By random use of the four transformations involved in lattice closure, this lattice is transformed into a closed cage containing 12 pentagons and two hexagons ($12p + 2h$). Similarly, we may transform lattices with $N_{6,e} = 4$ or 5. The lattice with $N_{6,e} = 4$ yields a cage containing $12p + 14h$, whereas the lattice with $N_{6,e} = 5$ gives

Fig. 7. Transformation of regular lattices

Three regular lattices ($N_6 = 19, 37$ and 61) can be closed by using H_c and F transformations. The cages formed by closure can be further transformed by using one H_c transformation per edge of the cage. This relaxation of curvature can be used to account for the apparent random distribution of pentagons in the final cage structure and the absence of septagons.

rise to a cage containing $18p + 20h + 6s$. If closure is attempted by using lattices with $N_{6,e} > 5$, complete closure is not possible. Hub-centre transformation must further be employed to allow for their closure. Thus we have chosen to work only with lattices that can be closed by using H_c and F transformations, making the study of such transformation as simple as possible.

In two of the three cages generated ($12p + 14h$ and $18p + 20h + 6s$), further transformation can be employed to relax the severely localized curvature generated by closure. 'Relaxation' of this curvature must be accomplished by using H_c transformation, since cages only contain polygons in configuration (a), i.e. four polygons connected by two vertices. By using one H_c transformation [$6, (5, 5), 7 \rightarrow 5, 6, 6$, or $6, (5, 5), 6 \rightarrow 5, 6, 5]$ per 'edge' of the cage, the curvature can be more evenly and symmetrically spread. (Since it is difficult to represent three-dimensional objects on a two-dimensional surface, we have opted to represent the clathrin cages using a form of distorted representation. The 'edge' of a cage is actually the side of a hexagon forming the bottom of the cage that is hidden from view in electron micrographs. This bottom hexagon, which appears to be an edge in distorted representation, is closest to the plasma membrane, and the central core hexagon is the furthest polygon from the plasma membrane.) This may be used to explain the sudden uniform appearance of pentagons in all areas of the coated vesicle. Pentagons rarely are seen in electron micrographs of closing lattices, and the observed final coated vesicles have a uniform distribution of pentagons (Heuser, 1980). This apparent jump from an absence of pentagons to a visibly uniform distribution of pentagons can be now explained, since the pentagons formed during closure would be hidden from view in Heuser's (1980) electron micrographs, and only after the 'relaxation of curvature' by using H_c transformation would they become visible.

Many-hexagon cores yield irregular lattices and their corresponding cages

In order to generate additional lattices and cages, we began to experiment with cores that contained more than a single hexagon (Fig. 8). After identifying all possible cores for lattice construction, we proceeded to add concentric rings of hexagons and observed the variable $N_{6,e}$. Since we have restricted our study to lattices that contain only $N_{6,e} = 3, 4$ or 5 , only cores and their corresponding lattices conforming to this restriction were chosen for illustration.

In these irregular lattices, the $N_{6,e}$ may vary from edge to edge (of which there are always six). Since these lattices differ from the regular lattices

Fig. 8. Many-hexagon cores used in the construction of irregular lattices

Concentric rings of hexagons were built around these cores and the $N_{6,e}$ was observed. Only lattices with all the $N_{6,e} = 3, 4$ or 5 were chosen for transformation.

Fig. 9. Transformation of lattice cages

The $N_{6,e}$ determines the final configuration after closure by means of H_c and F transformations. Therefore the cage produced by using only H_c and F transformations can be specified by knowing the $N_{6,e}$ for each edge of the lattice. For closure by means of H_c and F transformations, only $N_{6,e} = 3, 4$ or 5 may be utilized.

previously described, only eqn. (7), the formula for the number of hexagons present around the periphery ($N_{6,p}$), applies to both regular and irregular lattices.

Cores containing from two to five hexagons were found to give rise to suitable lattices. After identifying all such lattices, we proceeded to close them by using the generalization derived from regular hexagonal lattices: each edge is independent of the next and gives rise to a specific configuration only dependent on the $N_{6,e}$ (Fig. 9). In all cases, this leads to conversion of the ring of hexagons, once removed from the most peripheral

Fig. 10. *Irregular lattices containing many-hexagon cores and their transformation into cages*
 Lattices with cores containing two to five hexagons were found to be suitable for closure by means of H_c and F transformations. (a) Di-hexagonal core lattices; (b) linear tri-hexagonal core lattices; (c) triangular tri-hexagonal core lattices; (d) tetra-hexagonal core lattices; (e) penta-hexagonal core lattices.

ring, into pentagons and closure of the lattice. The severely localized curvature generated by favouring closure over curvature (by using only F and H_c) can be relaxed as with regular lattices by using one H_c transformation per edge of the cage. All eight lattices with their two corresponding cages (one from F and H_c , the other from F , H_c and H_c) are illustrated here, with their specific polygonal compositions (Fig. 10).

Does the model correlate well with observed cage structures?

Having now generated a total of 11 lattices and their corresponding cages, we may now specify which hexagonal lattice may give rise to a specific cage and even predict the three-dimensional configuration of the final cage. Since the three-dimensional configuration gives us the N_5 , N_6 and

N_7 in a cage, we may also calculate its size and attempt to correlate both size and configuration with experimental observation.

According to Table 1, our lattices range from 147.4 nm to 264.2 nm in diameter, whereas cages range from 53.4 to 107.4 nm in diameter. Since most coated vesicles fall in the range of 50–120 nm in diameter (Steven *et al.*, 1983), our model has successfully accounted for the selective size range of coated vesicles in disparate tissues. The gradual increase in size from one cage to another justifies the continuity in size range observed in populations of coated vesicles.

Several additional experimental observations lend support to the validity of our model. First, Heuser (1980) reports that for the fibroblast the average number of hexagons present in a lattice is 61 ($N_6 = 61$) and that the average size of a coated

Table 1. *Clathrin lattices (L) and cages (C,1 and C,2)*
 The radii of the lattices were calculated by using $R_L = [(N_6 A_6)/\pi]^{\frac{1}{2}}$, and the radii of the cages were calculated by using $R_C = [(N_5 A_5 + N_6 A_6 + N_7 A_7)/4\pi]^{\frac{1}{2}}$, where $A_5 = 595.2 \text{ nm}^2$, $A_6 = 898.8 \text{ nm}^2$ and $A_7 = 1257.2 \text{ nm}^2$.

Case	L			C,1				C,2		
	N_6	$N_{6,p}$	R_L	N_5	N_6	N_7	$R_{C,1}$	N_5	N_6	$R_{C,2}$
1	19	12	73.7	12	2	0	26.7	—	—	—
2	24	14	82.9	12	5	0	30.4	12	3	28.0
3	27	15	87.9	12	7	0	32.7	12	4	29.2
4	29	16	91.1	14	4	2	33.9	12	6	31.6
5	30	16	92.6	12	9	0	34.8	12	5	30.4
6	33	17	97.2	13	9	1	36.9	12	7	32.7
7	37	18	102.9	12	14	0	39.6	12	8	33.8
8	44	20	112.2	14	15	2	44.0	12	13	38.7
9	48	21	117.2	15	16	3	46.4	12	16	41.4
10	52	22	122.0	16	17	4	48.7	12	19	43.9
11	61	24	132.1	18	20	6	53.7	12	26	49.3

vesicle is 100nm in diameter. In case 11 of our model, a lattice containing 61 hexagons is transformed into a cage with a diameter of 98.6nm: a difference of only 1.4nm. [Our calculated diameter was based on two assumptions: (a) that the polygons that make up the clathrin cage are regular; (b) that the area of the cage can be approximated by the area of a sphere. To test the validity of these assumptions, a comparison was made between the observed diameter of the final cage produced in case 7 and the calculated diameter. An experimental diameter of 65nm was reported by Pearse & Robinson (1984), whereas we calculated a diameter of 67.6nm. Since the difference between the experimental and calculated diameters is only 2.6nm, the difference of 1.4nm observed for case 11 and the average-size fibroblast coated vesicle is well within the error inherent in our approximation.] Secondly, the final cage structures produced by our model for cases 3 and 7 are identical with the structures of the smallest brain coated vesicles reported by Crowther *et al.* (1976); further suggesting that our model is an adequate representation of the process of the formation of coated vesicles *in vivo*. Finally, it is worth noting that all the final cages contain the correct number of pentagons ($N_5 = 12$) and septagons have been eliminated, which is exactly what is observed experimentally (Heuser, 1980).

It should be noted that, although our model can be used to account for two of the three structures proposed for the simplest coated vesicles by Crowther *et al.* (1976), it cannot, as yet, be used to generate the other structure of coated vesicles. Since we have not attempted to enumerate all the possible lattices, other lattices may be found that

may give rise to this configuration of coated vesicles. For example, additional lattices may be generated by using purely pentagonal cores, or cores containing a mixture of hexagons and pentagons. Alternatively, this structure may be the product of re-arrangements that have occurred during the isolation procedure, since the high concentrations of sucrose employed during isolation have been found to promote the dissociation of clathrin-coated vesicles (Nandi *et al.*, 1982).

Conclusions

We have presented a mathematical model of the formation of coated vesicles that allows us to trace the intricate sequence of geometrical transformations involved in creating a coated vesicle. This scheme of closure, using F and H_c transformations, followed by relaxation of curvature, using H_c transformation, can be used to generate a whole spectrum of coated vesicles. It is only the initial geometry of the flat hexagonal lattice that is needed to specify the final configuration. Such a simple geometrical solution to an apparently complex problem argues strongly in its favour as the correct solution.

Although our model is obviously a simplified explanation for the formation of coated vesicles, it provides a working hypothesis that integrates a great deal of seemingly disparate experimental observations. It also demonstrates the importance of geometrical constraints that must be kept in mind when investigating the process of the formation of coated vesicles, as it is essentially a geometrical transformation process.

This study was supported in part by National Institutes of Health Grant NS12467 to S. P. We thank Dr. Gary Findley, Graduate Department of Physical Chemistry, and Dr. Charles Peskin, the Courant Institute of Mathematical Sciences, New York, University, for critical discussions, Debra Heitmann for illustrations, and Kathleen Tannuzzo and John Morgan for editing.

References

- Braell, W. A., Schlossman, D. M., Schmid, S. L. & Rothman, J. E. (1984) *J. Cell Biol.* **99**, 734–741
- Crowther, R. A. & Pearse, B. M. F. (1981) *J. Cell Biol.* **91**, 790–797
- Crowther, R. A., Finch, J. T. & Pearse, B. M. F. (1976) *J. Mol. Biol.* **103**, 785–798
- Heuser, J. (1980) *J. Cell Biol.* **84**, 560–583
- Katsura, I. (1983) *J. Theor. Biol.* **103**, 63–75
- Keen, J. H., Willingham, M. C. & Pastan, I. H. (1979) *Cell (Cambridge, Mass.)* **16**, 303–312

- Kirchhausen, T. & Harrison, S. C. (1981) *Cell* (Cambridge, Mass.) **23**, 755–761
- Lisanti, M. P., Schook, W., Moskowitz, N., Beckenstein, K., Bloom, W. & Puszkin, S. (1982a) *Eur. J. Biochem.* **121**, 617–622
- Lisanti, M. P., Schook, W., Moskowitz, N., Ores, C. & Puszkin, S. (1982b) *Biochem. J.* **201**, 297–304
- Lisanti, M. P., Sharpiro, L., Moskowitz, N., Hua, E., Puszkin, S. & Schook, W. (1982c) *Eur. J. Biochem.* **125**, 463–470
- Lisanti, M. P., Flanagan, M. & Puszkin, S. (1984) *J. Theor. Biol.* **108**, 143–157
- McKinley, D. M. (1983) *J. Theor. Biol.* **103**, 405–419
- Nandi, P. K., Igrace, G., VanJaarsveld, P. P., Lippoldt, R. E. & Edelhoach, H. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5881–5885
- Pearse, B. M. F. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1255–1259
- Pearse, B. M. F. & Robinson, M. S. (1984) *EMBO J.* **3**, 1951–1957
- Schlossman, D. M., Schmid, S. L., Braell, W. A. & Rothman, J. E. (1984) *J. Cell Biol.* **99**, 723–733
- Schmid, S. L., Matsumoto, A. K. & Rothman, J. E. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 91–95
- Schook, W., Puszkin, S., Bloom, W., Ores, C. & Kochwa, S. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 116–120
- Steven, A. C., Hainfeld, J. F., Wall, J. S. & Steer, C. J. (1983) *J. Cell Biol.* **97**, 1714–1723
- Unanue, E. R., Ungewickell, E. & Branton, D. (1981) *Cell* (Cambridge, Mass.) **26**, 439–446
- Ungewickell, E. & Branton, D. (1981) *Nature* (London) **289**, 420–422
- VanJaarsveld, P. P., Nandi, P. K., Lippoldt, R. E., Saroff, H. & Edelhoach, H. (1981) *Biochemistry* **20**, 4129–4135