STATISTICAL MECHANICAL MODEL OF THE ENERGETICS OF COATED VESICLE FORMATION

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ABSTRACT Adsorptive endocytosis is mediated by a structural transformation of a two-dimensional hexagonal lattice to a polyhedron or coated vesicle. Clathrin is the structural protein involved in this process. A theoretical model that focuses on the energetics of clathrin in coated vesicle formation is presented. Fisher's cluster model of phase transitions is applied to this problem. The equilibrium constant for the process of converting a large two-dimensional patch to a coated vesicle and a smaller patch is calculated. There are three energetic contributions to be considered. They are the surface energy, the interior or lattice energy, and a loop entropy. Features of the Ising model are introduced into this model by scaling the critical exponents. In determining the configurational partition functions required for equilibrium constant calculations, the polyhedron is represented as a planar graph with no surfaces. The equilibrium constants are extremely sensitive to changes in the lattice and surface energies. The loop entropy contributions favor bimodal distributions in vesicle size under certain conditions. Conditions can also be established where the energy required to form the vesicle is small.

INTRODUCTION

Recent findings suggest that cellular transport of macromolecules by coated vesicles is a common and general phenomenon in eukaryotes (see reference 1). In adsorptive endocytosis a patch of receptors on the cell surface is transformed into a vesicle in the interior of the cell. The structural protein involved in this process is called clathrin. Clathrin forms an amazingly regular hexagonal lattice in the membrane (2). In 1981, Ungewickell and Branton observed by electron microscopy that the polyhedral units were constructed of units they termed "triskelions" (3). Each triskelion consists of three clathrin molecules joined together at one vertex. The clathrins form bent arms leading out of the vertex, giving the triskelion a "pinwheel" appearance. Specific receptors for the molecules to be endocytosed bind to the clathrin network. The endocytotic process involves a structural transformation of the clathrin network from a two-dimensional hexagonal lattice into a polyhedron made up of twelve pentagons and a variable number of hexagons. The energetics of this process is unknown. However, it has been suggested that this structural transformation could occur spontaneously by a series of dislocations in the two-dimensional lattice (4). Another possibility is that actin filaments could serve to pull the clathrin network into the cell. A subsequent pinching of the membrane at the cell surface would form the coated vesicle. Actin filaments have been observed near the membrane surface (2). There is clearly not enough experimental evidence to favor one model over another.

In this paper we develop a statistical mechanical model to assess the various energetic contributions to this structural transformation. We focus solely on the clathrin network considering only the transformation of a two-dimensional lattice into a polyhedral structural. Our model is based upon Fisher's "cluster" or "droplet" model of phase transitions (5, 6). It incorporates the features of a two-dimensional Ising model by a renormalization of critical exponents. These exponents depend primarily on the dimensionality of the cluster. The application of this theory to the clathrin structural transformation allows a general assessment of some of the factors that favor coated vesicle formation. Conditions are also established that yield a bimodal population distribution in vesicle size. Similar bimodal distributions have been observed under physiological conditions (7) and in vitro (8).

THEORY

The problem of the energetics of coated vesicle formation is modeled by the transformation of a two-dimensional lattice into a polyhedron. Thus, the following process is considered:

$$\text{Two-dimensional lattice of } M \text{ points} \rightleftharpoons \text{polyhedron of } N \text{ points} + \text{two-dimensional lattice of } M - N \text{ points.} \quad (1)$$

This process is illustrated in Fig. 1. The two-dimensional lattice on the cell surface tends to be hexagonal with three clathrins meeting at each vertex and three clathrins forming an edge. In this model we consider the energetics of the vertices and not the individual clathrins. The polyhedrons consist of 12 pentagons and a variable number of hexagons as determined by electron microscopy (2, 9). Relationships between the number of edges, vertices, and faces can be derived from Euler's equation and elementary equations of graph theory. It can be easily shown that for a polyhedron whose vertices are all third order (three edges) and whose faces are either pentagonal or hexagonal there must be 12 and only 12

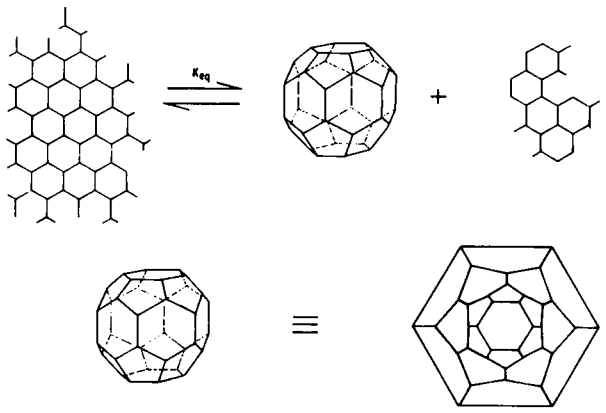

FIGURE 1 A pictorial representation of an equilibrium process in which the planar, hexagonal lattice of clathrin is transformed into a polyhedron or vesicle and a smaller planar lattice. The isomorphism of a polyhedron with a planar graph is shown.

pentagons and N must be an even number. The smallest possible number for N is 22. When calculating an equilibrium constant for the process in Eq. 1, one must bear in mind these restrictions on N. As will become evident from the theory, the details of the lattice and polyhedron structure will not be as important as their dimensionality.

To analyze this situation an application of the Fisher cluster or droplet theory is used. This theory has been used to demonstrate the effects of short range interactions and dimensionality on critical phenomena (5, 6). In this microscopic model, any configuration of particles is considered to be composed of a collection of clusters. Each cluster consists of an integral number of particles in close association. Particles within the cluster interact in pairs, and clusters are not considered to interact with each other. This emphasizes the short-range nature of the interactions. The dimensionality of the system is introduced through scaling laws. These laws relate parameters of the model to critical exponents. The critical exponents are obtained either experimentally or for two dimensions by an exact solution of the Ising model.

The Fisher theory allows the calculation of a configurational partition function, q_l for a cluster of l particles. This is related to the total energy of the cluster by

$$\Delta G(l) = -RT \ln(q_l/V). \tag{2}$$

The cluster free energy, $\Delta G(l)$, is divided into three terms; an interior or bulk energy term, a surface energy term, and a loop entropy term. This gives

$$\Delta G(l) = \Delta G_{\text{INTERIOR}} + \Delta G_{\text{SURFACE}} + \Delta G_{\text{LOOP}} \tag{3}$$

where

$$-\frac{\Delta G_{\text{INTERIOR}}}{RT} = \frac{l\phi}{RT} = l \ln y$$

$$-\frac{\Delta G_{\text{SURFACE}}}{RT} = \frac{a_0 l^{\sigma}\gamma}{RT} = l^{\sigma} \ln x$$

$$-\frac{\Delta G_{\text{LOOP}}}{RT} = \tau \ln l - \ln q_0.$$

The interior energy term, ϕ, represents the lattice binding energy of the three clathrins making up a vertex located inside the cluster. The surface term is due to the lost clathrin-clathrin interactions at the edge of the cluster. It consists of a surface tension, γ, multiplied by the surface area, $a_0 l^{\sigma}$. For two dimensions $\sigma = 0.533$, but for three dimensions $\sigma = 0.640$. On the basis of simple geometric considerations of surface areas in two and three dimensions, $\sigma \simeq 0.5$ and 0.667, respectively. The slightly higher values determined by the scaling laws is presumably due to the fact that the surface can be uneven and can have ripples in it. The loop energy term is an entropy effect. It is due to the restriction of the number of possible configurations of a cluster by the fact that the surface must close on itself. Thus, a random walk over the surface of the cluster must always be capable of returning to its starting point. The form of this entropy contribution was determined from well known relationships concerning random lattice walks. In two dimensions $\tau = 2.067$ and is the same for a square or triangular lattice. In three dimensions $\tau = 2.192$. The parameter τ like σ is determined from scaling laws for the cluster model (5). The term, q_0, is an amplitude factor for the loop entropy. In a previous application of the cluster model to lipid phase transitions q_0 was calculated by (10)

$$q_0(l) = \tfrac{1}{2}\left[\sum_{i=1}^{l} i^{(1-\tau)}\right]^{-1}. \tag{4}$$

This is determined by the requirement the $\Delta G_{(\text{TOTAL})} = 0$, $x = 0$, and $y = 0$ at a critical temperature.

The configurational partition function for a cluster is then given by

$$\frac{q_l}{V} = \frac{q_0(l)x^l y^l}{l^{\tau}} = e^{-\Delta G(l)/RT}. \tag{5}$$

Defining the equilibrium constant for the process in Eq. 1 as

$$-RT \ln K_{\text{eq}} = \Delta G_N(\text{vesicle}) + \Delta G_{M-N}(2D \text{ patch}) - \Delta G_M(2D \text{ patch}) \tag{6}$$

and using Eqs. 4–6 gives

$$K_{\text{eq}} = \frac{q_{M-N}(2D \text{ patch})\, q_N(\text{vesicle})}{V\, q_M(2D \text{ patch})}. \tag{7}$$

In the calculation of q_l for the hexagonal two-dimensional lattice, the appropriate values of σ and τ are 0.533 and 2.067, respectively. In considering the configurational partition function for the vesicle or polyhedron, there are two major simplifying factors. First, there is no exposed surface with unbound clathrin ends. Therefore, the surface energy is nonexistent and $x = 1$. It doesn't matter what the value of σ is in this case. The second simplification results from the fact that all polyhedron are topologically isomorphic with a planar graph. A polyhedron is considered to be an "embedding" of a planar graph. This isomorphism is illustrated in Fig. 1. Thus, the appropriate value for τ is the two-dimensional value of 2.067. Taking these factors into consideration the equilibrium constant becomes

$$K_{\text{eq}} = \frac{q_0(M-N)q_0(N)}{q_0(M)}\left(\frac{M}{(M-N)(N)}\right)^{\tau} x^{(M-N)^{\sigma}-M^{\sigma}} R^N \tag{8}$$

where $R = y_{(\text{vesicle})}/y_{(2D \text{ patch})}$. If the clathrin-clathrin bonds are strained in going from the patch to the vesicles then $R < 1$. If there is no strain, then $R \simeq 1$.

In this calculation the translational energy has not been accounted for. There are two contributions to be considered. The first is the translation of vertices within the lattice. This will have no effect on the equilibrium constant since both the patch and the vesicles can be viewed as two-dimensional structures. The second contribution is due to the translation of the entire patch on the cell surface and to the translation of the vesicle in the interior of the cell. Because the vesicle has one more degree of translational freedom, the overall effect would favor vesicle formation. A calculation of the translation partition functions would require an estimate of the entire mass that moves with the patch and of the mass of the contents of the vesicle. We do not consider these terms, although they

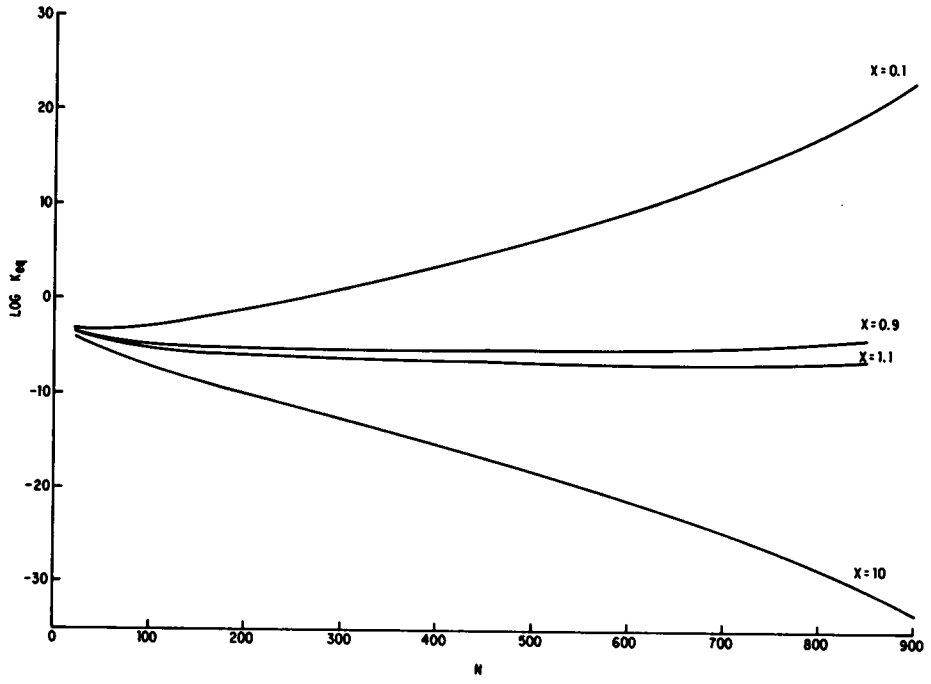

FIGURE 2 A plot of log K_{eq} vs. N, the number of vertices in the polyhedron. The total number of vertices is 1,000. Conditions are given where the surface energy of the planar patch is favorable, $x > 1$, and unfavorable, $x < 1$. The internal lattice energy is constant, $R = 1$.

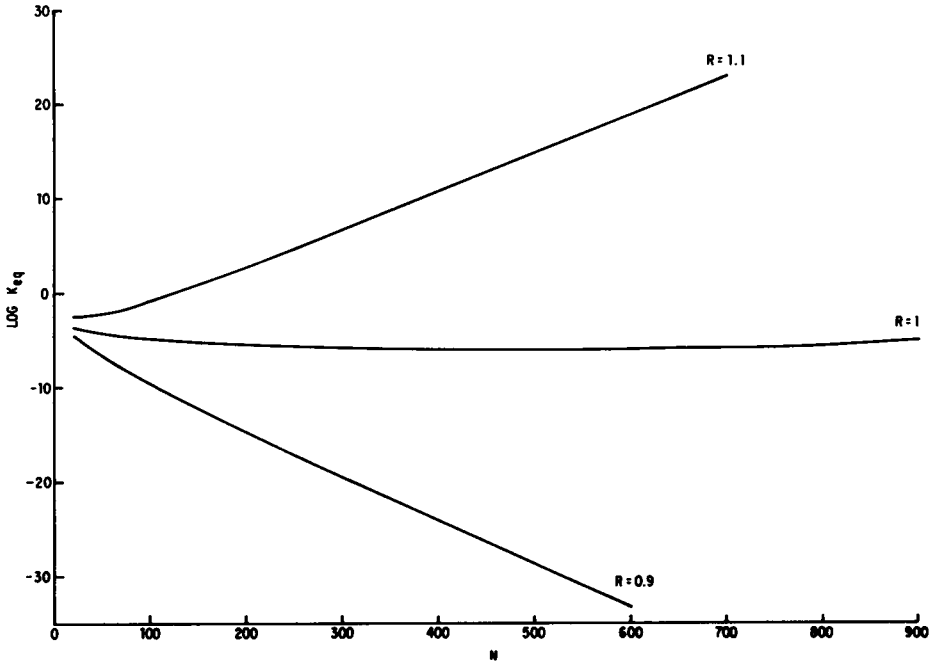

FIGURE 3 A plot of log K_{eq} vs. N, the number of vertices in the polyhedron. The total number of vertices is 1,000. Conditions are given where the internal lattice energy changes from the planar patch to the polyhedron. For $R < 1$ the internal energy is less favorable in the polyhedron and for $R > 1$ it is more favorable. The surface energy contribution is set equal to zero, $x = 1$.

may have a significant effect. Given reasonable estimates of the appropriate masses, these contributions could be easily accounted for.

RESULTS AND DISCUSSION

Using Eq. 8, the effects of the three energy terms specified in Eq. 2 can be demonstrated. Fig. 2 shows the effect of the surface energy. In this figure, K_{eq} vs. N is given for several different patch surface energies. A slight unfavorable unit surface energy, $x < 1$, will strongly favor vesicle formation, while a slightly favorable surface energy, $x > 1$, will strongly disfavor it. In Fig. 3, the effects of differences in the interior lattice energy are shown. A small change in the lattice strain has profound effects on the equilibrium constant. The loop entropy contributions are shown in Fig. 4. These curves show that vesicle formation is enhanced for both small and very large N. The effects are qualitatively independent of patch size, M. The loop entropy is most favored when the original patch breaks down to a second large structure, regardless of whether this be another patch (small N) or a large vesicle (large N).

In Fig. 5, K_{eq} vs. N is shown under conditions that are closer to the physiological case. In this plot, R is slightly <1 indicating a small strain in the clathrin-clathrin interaction. x is expected to be relatively small due to the lost clathrin-clathrin interactions at the surface. Fig. 5 indicates that these two effects can counteract each other to some extent. The resulting equilibrium constants then favor vesicles of very small or very large N.

Application of the cluster model to the structural transformation of a clathrin network allows a simple visualization of some of the energetic factors involved in coated vesicle formation. As has been shown, a slight strain in the clathrin-clathrin interactions in the polyhedron can have an extremely large unfavorable effect on the process. These forces are probably counterbalanced by the unfavorable surface interactions in the patch. An experimental determination of the thermodynamics of clathrin-clathrin interactions would allow an estimate of the unfavorable surface energy caused by "lost" clathrin-clathrin interactions. One example in Fig. 5 ($R = 0.4$, $x = 1.0 \times 10^{-14}$) shows that a strain of ~0.5 kcal/vertex at room temperature is more than compensated at low and high vesicle size by an unfavorable energy of 19 kcal for a surface vertex. Most surface vertices will have two free clathrin ends. If a clathrin-clathrin interaction is ~10 kcal then this would be a realistic number for a surface energy. The interesting feature of this model is that it is not difficult to have conditions in which a bimodal distribution consisting of very small and very large vesicles is possible. This effect is due primarily to the loop entropy. Such bimodal distributions have already been observed experimentally (7, 8).

In considering the energetics of the process, the model of spontaneous transformation (4) cannot be ruled out. It is not unusual to have favorable equilibrium constants, especially at high N. However, we frequently observe equilibrium constants between 10^{-3} and 10^{-4} at low N. This

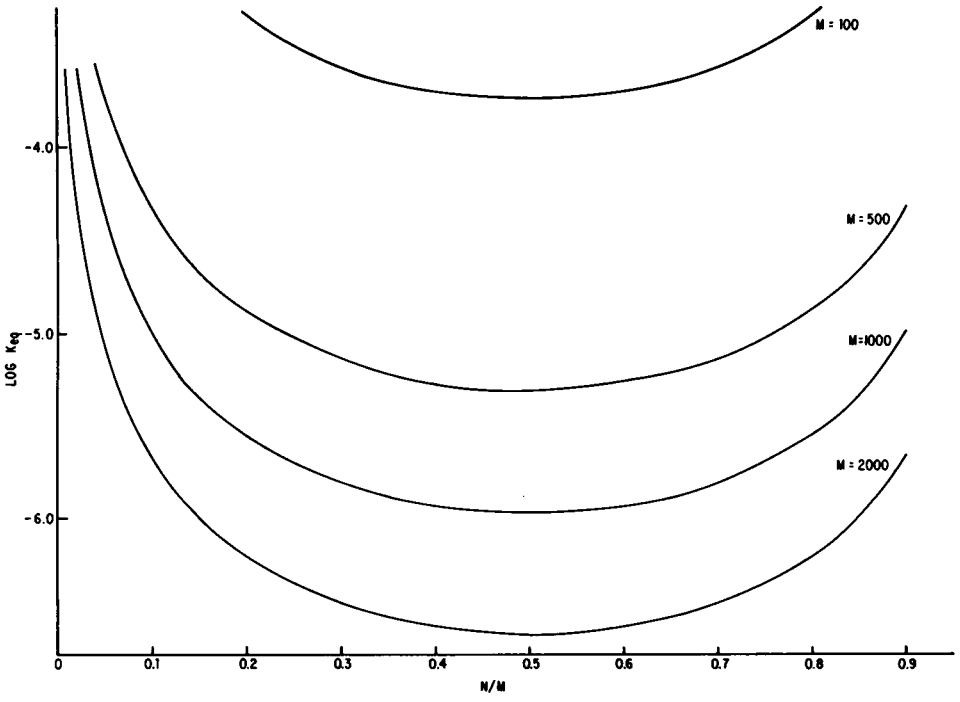

FIGURE 4 A plot of log K_{eq} vs. N/M, the ratio of the number of vertices in the polyhedron to the total number of vertices. Conditions are given where the surface energy is zero and there is no change in the internal lattice energy. These plots demonstrate the contributions from the loop entropy. The dependence on total number of vertices is shown.

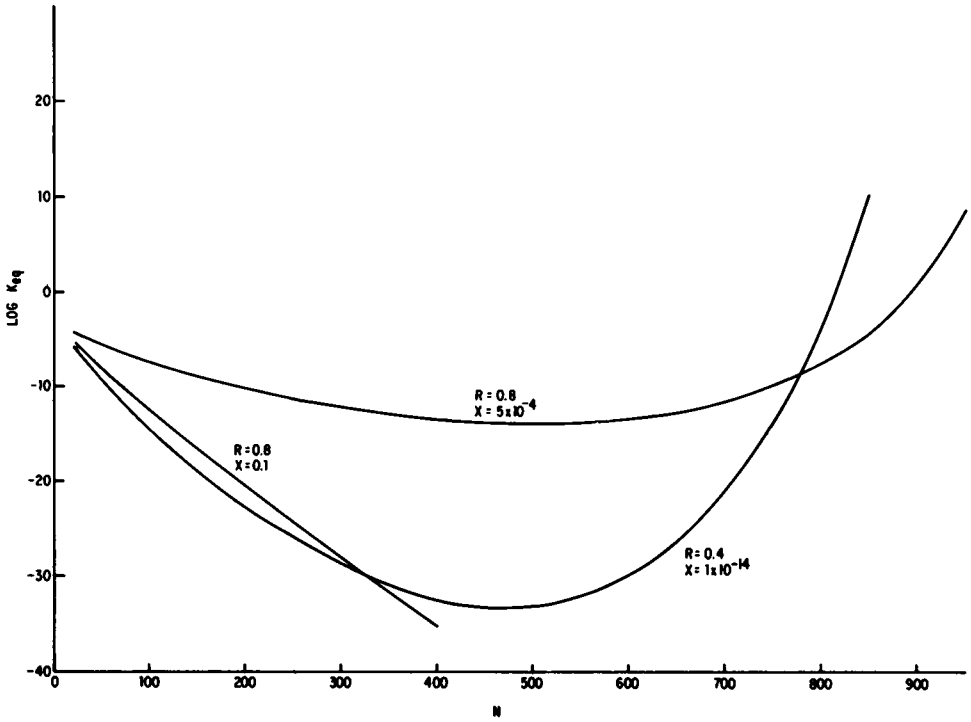

FIGURE 5 A plot of log K_{eq} vs. N, the number of vertices in the polyhedron. The total number of vertices is 1,000. Conditions are given where the polyhedron has a slightly less favorable internal lattice energy than the planar patch. The planar patch has an unfavorable surface energy contribution due to lost clathrin-clathrin interactions. These effects are shown to counterbalance each other under certain conditions.

would suggest that a small amount of energy, e.g., the hydrolysis of one ATP, would be required to drive the transformation. While this cluster model is undoubtedly an oversimplification of the overall energetics of coated vesicle formation, it can provide some guidelines to the factors important in this process.

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