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A MOLECULAR MODEL FOR VESICLE FORMATION

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A molecular model is proposed to explain vesicle formation. The model is based on a balance between elastic and 'hydrophobic' forces for various micelle and bilayer geometries in dilute aqueous solutions. It predicts bilayered disc-like transition structures and is in agreement with experimental data.

The exact mechanism of vesicle formation is not known. The understanding of this process on the molecular level would be helpful for the preparation of vesicles, especially by the detergent-removal method where vesicles are formed from mixed detergent/diacyl lipid micelles after the removal of detergent by either dialysis [1], chromatography [2] or treatment with detergent absorbers [3]. Many experiments using detergent-removal techniques only yield empirical conclusions. For example, octylglucoside treatment produces large vesicles [4] whereas cholate gives rise to small vesicles [2], while the use of many other detergents does not even produce vesicles. However, there is little information available concerning intermediate structures or the mechanism of the formation of vesicles from mixed micelles.

The energetic considerations of different states of diacyl lipid molecular aggregates in dilute aqueous solutions have been previously discussed [5–8]. In dilute aqueous solutions, most diacyl lipids can form either infinite, flat-bilayered lamellae, closed vesicles, or intermediary structures, i.e., curved lamellae.

In the case of flat lamellae, the interaction energy of the contact of the non-polar hydrocarbon chains with water, E_h, is thermodynamically unfavoured, since:

$$E_h = \gamma S$$

here S is the circumference of the polar/non-polar boundary, with a circle having minimal circumference for a fixed area and γ is the boundary interaction energy per unit length [5]. The system tends to minimize E_h and begins to curve, decreasing S, but increasing the elastic curvature energy, E_c:

$$E_c = \tfrac{1}{2} kA/R^2$$

where k is elastic modulus, A the surface area of a lamella and R the mean radius of curvature [5]. E_c increases until the bilayered lamella forms a closed vesicle where a constant value $2\pi k$ is obtained. At this point $S = 0$ and therefore $E_h = 0$. With Hookean approximation, the parabolic elastic energy, E_p, due to the compression and decompression of the polar head group area is neglected for $R > R_c$, the critical radius, which is approx. 10 nm for egg yolk lecithin vesicles [7]. Then, assuming that all the other energy contributions are equal for all possible shapes of aggregates, the shape will be determined only by the balance of E_h and E_c at $R > R_c$. For small curvatures, the total excess energy may be expressed as a function of the number

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of molecules N in the structure by:

$$E(N,R) = E_p(N,R) + \gamma(2a\pi)^{1/2}N^{1/2}(1 - Na/8\pi R^2)^{1/2} + \tfrac{1}{4}\,kNa/R^2$$

where a is the area of the polar head group.

With this model the formation of vesicles by various preparation techniques, especially by the detergent-depletion method, can be explained. When vesicles are being prepared by the detergent-removal technique, we assume that mixed detergent/lipid micelles are growing through collision-induced fusions into flat, disc-like micelles, because this reduces the total S of the system. There is some phase separation in the micelles [9]. The small detergent molecules are concentrated at the disc edges where they shield the non-polar acyl chains of the lipid from water [10]. This shielding reduces the value of E_h. With continuing removal of detergent, the flat micelles continue to fuse reducing the total circumference of the shielded non-polar/polar interface. (This is in agreement with quasi-elastic light-scattering and electron-microscopy data which show that the size of mixed micelles increases with decreasing concentration of detergent [10].) The bilayered disc-like micelles with radius $r < 2R_c$ will not curve, because the non-Hookean elastic energy, E_p, due to the change of a:

$$E_p = \tfrac{1}{2}k_p\,\Delta a^2/a^2$$

is $E_p > E_h$. When the micelle grows over the critical area and when there is not enough detergent to shield the exposed circumference, the system begins to curve, i.e., decreasing S, and finally vesiculates.

The shielding can be more or less effective, dependent on the detergent molecular geometry and its properties. For example, octylglucoside is very effective at covering hydrophobic areas due to its geometry as well as ability to make a cover with high curvature. Therefore, it can shield the boundary much more effectively than molecules of rigid sodium cholate. Some detergents do not produce vesicles because shielding is ineffective or because their molecules do not migrate to the disc edges.

The shape of a molecular aggregate without detergent is dependent on the values of E_h and E_c. In the presence of detergent one may define an additional term, E_s, which is due to the shielding effect of detergent and which decreases E_h. Without detergent and with $R > R_c$, the flat disc is thermodynamically unstable when $E_h > E_c$. With detergent this condition becomes $(E_h - E_s) > E_c$, and larger flat structures can be formed. Empirically, the value of γ in E_h can be replaced by the effective value, $\gamma_{eff} = f\gamma$. Shielding is included in the factor f, where $0 \leqslant f \leqslant 1$. Helfrich [5] obtained an expression for vesicle diameter $2R_0 = 4k/\gamma$ by minimizing the total energy with respect to the curvature, $d(E_c + E_h)/d(1/R) = 0$. Using $k = 2.3 \cdot 10^{-12}$ erg [11] and adjusting [5] $\gamma = 7.2 \cdot 10^{-6}$ erg/cm with respect to the diameters of sonicated vesicles [2,12], one obtains $f = 0.07$ for octylglucoside and $f = 0.52$ for sodium cholate.

Assuming this mechanism of vesicle formation, the vesicle size, unilamellar nature, and homogeneity of detergent-removal preparations can be understood.

According to this model, the orientation of molecules embedded in the bilayer of large vesicles should be random. Only in the case of very small vesicles should non-symmetric enzymes be oriented due to steric or, in the case of charged groups, electrostatic interactions in the small inner aqueous space of the vesicle.

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