Hypothesis

Coupling between vesicle shape and the non-homogeneous lateral distribution of membrane constituents in Golgi bodies

Aleš Iglič[a,*], Miha Fošnarič[a], Henry Hägerstrand[b], Veronika Kralj-Iglič[c]

[a] *Laboratory of Applied Physics, Faculty of Electrical Engineering, University of Ljubljana, Tržaška 25, SI-1000 Ljubljana, Slovenia*
[b] *Department of Biology, BIOCITY, Åbo Akademi University, FIN-20520 Åbo/Turku, Finland*
[c] *Institute of Biophysics, Faculty of Medicine, Lipičeva 2, SI-1000 Ljubljana, Slovenia*

Received 29 June 2004; accepted 27 July 2004

Available online 14 August 2004

Edited by Sandro Sonnino

Abstract In this work, a hypothesis is presented that could explain the non-homogeneous lateral distribution of membrane components in Golgi vesicles. It is shown that the non-homogeneous lateral distribution of membrane components and the specific flattened shape of Golgi vesicles are strongly coupled. In agreement with experimental evidence, it is indicated that some of the membrane components may be concentrated mainly on the curved bulbous rims of the Golgi vesicles, while the other components are distributed predominantly in their flat central part.

Keywords: Golgi body; Non-homogenenous lateral distribution; Anisotropic membrane component; Vesicle shape; Rafts

1. Introduction

In Golgi bodies small spherical daughter vesicles are pinched off predominantly from the curved rims of the large flattened Golgi vesicles [21,26]. These protein-coated carrier vesicles (also called COPI vesicles) are involved in transport through the movement of Golgi bodies up and down the Golgi stack and also in transport from Golgi bodies to endoplasmic reticulum (ER) [26]. Although Golgi bodies and ER are connected by bidirectional vesicular transport, they maintain distinct membrane compositions [3,26]. The lipid composition of the membrane gradually changes along the stack of Golgi vesicles from ER (60% phosphatidylcholine, 25% phosphatidylethanolamine and 10% phosphoinositol) to that of plasma membrane (25% phosphatidylcholine, 15% phosphatidylethanolamine, 30–40% cholesterol, 10% sphingolipids and 5% phosphatidylserine) [26]. This indicates that vesicular transport between cell organelles is not random [26] and takes place only between specific surface regions of the cell organelles. Such organized transport may be achieved either by variation of the lateral concentration of the membrane components that may favor the formation of the thin necks (with very high anisotropic curvature) connecting the daughter vesicles with the mother membrane [11,13,18,26], and/or by direct transport through nanotubes or nanotube-directed transport of carrier vesicles [4,15,25], where the nanotubes are attached only in certain regions of the organelles' surface. Indeed, it has been indicated recently that at least for certain cargoes the Golgi-to-ER transport routes are COPI independent [3].

Further, the protein and lipid composition of the carrier vesicles budding from the Golgi bodies and fusing with the ER is not the same as the protein and lipid composition of the vesicles budding from ER and fusing with the Golgi bodies [26]. All this implies the lateral segregation of membrane lipids, i.e., the formation of lipid domains, and the non-homogeneous lateral distribution of membrane proteins [13,26] in the membrane of Golgi bodies. A non-homogeneous lateral distribution of membrane lipids has also been observed in lipid vesicles [10,12]. In red blood cells, a non-homogeneous lateral distribution of membrane lipids and proteins has been indicated [5,8].

Understanding the mechanism that maintains the non-homogeneous lateral distribution of lipids and proteins in biological membranes provides a major challenge in current cell biology and the precise mechanism by which it is accomplished remains to be established [13,26]. In this work, a possible physical mechanism maintaining the non-homogeneous lateral distribution of membrane proteins and lipids in Golgi vesicles is proposed. The coupling between the characteristic shape of Golgi vesicles with a thin plate-like central region and bulbous rim and the non-homogeneous lateral distribution of the membrane components is studied. It is shown that a non-homogeneous lateral distribution of the membrane components (proteins or lipids) is necessary for a Golgi vesicle to have its characteristic flattened shape.

2. Theory

Biological membranes are multi-component mixtures of phospholipids, cholesterol, glycolipids, lipoproteins, proteins and other components. In our theoretical model, we assume that the membrane is composed of an isotropic lipid bilayer in which different kinds of mobile inclusions are embedded. The inclusions may be membrane proteins, lipids, small lipid clusters or protein–lipid complexes [7,19,20,23]. Inclusions may in general be anisotropic [18]. For the sake of simplicity in this work all inclusions are ascribed to the outer lipid layer;

* Corresponding author. Fax: +386-1-4768-850.
E-mail address: ales.iglic@fe.uni-lj.si (A. Iglič).

0014-5793/$22.00
doi:10.1016/j.febslet.2004.07.085

however, it is not excluded that some types of inclusions (such as membrane integral proteins) may protrude through both lipid layers. Inclusions can move laterally over the phospholipid (bi)layer and also rotate around their axes normal to the membrane, so that they can find a place and orientation in the membrane that is energetically the most favorable [5,18]. Therefore, the equilibrium lateral distribution of inclusions in the membrane is in general non-uniform. In our model, the inclusions are treated as independent and indistinguishable [18]. The excluded volume effect is not taken into account. Interaction of inclusions with the surrounding membrane affects the free energy of the membrane.

A mismatch between the effective intrinsic shape of the inclusions and the actual shape of the membrane at the site of the inclusion gives rise to the single inclusion energy [6,18]. Starting from one inclusion, the equilibrium lateral distribution of inclusions is obtained by the methods of statistical physics. To obtain the expression for the free energy of all the membrane inclusions (F_{in}), the equilibrium lateral area distribution of the inclusions is inserted into the expression for the free energy of the inclusions and integrated over the whole membrane area A [18,19]:

$$F_{\mathrm{in}} = -\sum_{i=1}^{M} N_i kT \ln\left[\frac{1}{A}\int q_i I_0\left(\frac{\xi_i}{kT} D D_{\mathrm{m},i}\right)\mathrm{d}A\right], \qquad (1)$$

where $q_i = \exp(-\xi_i (H - H_{\mathrm{m},i})^2/2kT - \xi_i (D^2 + D_{\mathrm{m},i}^2)/2kT)$, ξ_i represents the strength of the interaction between an inclusion of type i and the surrounding membrane, M is the number of different types of inclusions, N_i is the total number of inclusions of type i, k is the Boltzmann constant, T is the absolute temperature, C_1 and C_2 are the two principal curvatures, $H = (C_1 + C_2)/2$ is the mean curvature of the membrane, $H_{\mathrm{m},i} = (C_{1\mathrm{m},i} + C_{2\mathrm{m},i})/2$ is the mean curvature describing the intrinsic shape of a single membrane inclusion, while $D = (C_1 - C_2)/2$, $D_{\mathrm{m},i} = (C_{1\mathrm{m},i} - C_{2\mathrm{m},i})/2$ are the curvature deviators and $\mathrm{d}A$ is the infinitesimal membrane area element. The intrinsic curvature deviator $D_{\mathrm{m},i}$ describes the intrinsic anisotropy of a single membrane inclusion [20]. In accordance with the definitions of C_1 and C_2, C_1 is always larger than C_2. This is consistent with the definition of the curvature deviator (D), which is always positive [20].

In order to determine the equilibrium lateral distribution of membrane inclusions and the corresponding equilibrium vesicle shape, the overall free energy of the vesicle membrane consisting of two terms,

$$F = \frac{1}{2} k_{\mathrm{c}} \int_A (2H - C_0)^2 \mathrm{d}A + F_{\mathrm{in}}, \qquad (2)$$

is minimized at constant volume (V) and area (A). The first term is the local membrane bending energy, i.e., the bending energy of the isotropic membrane bilayer without the inclusions. Here, k_{c} is the local bending modulus and C_0 is the spontaneous curvature [9]. The second term (F_{in}) describes the free energy of the inclusions.

In the calculations, analysis is restricted to axisymmetric vesicle shapes with equatorial mirror symmetry. The vesicle shape is parameterized by a function of the form [5]

$$y(x) = \pm\left(\alpha + \frac{(\gamma x)^{\delta}}{1 + (\gamma x)^{\delta}}\right)\sqrt{\beta^2 - x^2}, \qquad (3)$$

including four free parameters (α, β, γ and δ). The symmetry axis of the vesicle shape coincides with the y axis, so that the shape is given by rotation of the function $y(x)$ around the y axis. In the minimization procedure, the values of the free parameters that give the minimal free energy (F) are sought. Constraints requiring a fixed volume of the vesicle (V) and a fixed area of the vesicle membrane (A) are taken into account.

To estimate the values of the interaction constants of the inclusions, ξ_i, we consider a pure one-component isotropic lipid bilayer. The free energy of a one-component lipid bilayer can be simply derived using the single-molecule energy of the lipid molecules in the same form as used in the derivation of Eq. (1). If the lipid molecules are taken to be isotropic, then the average interaction constants of lipid molecules can be estimated as $\xi_i \approx k_{\mathrm{c}} a_0 \approx 10\ kT\mathrm{nm}^2$ [20], where $a_0 \approx 0.6\ \mathrm{nm}^2$ is the area per single lipid molecule and $k_{\mathrm{c}} \approx\ 20\ kT$ [22]. The interaction constants of larger and/or more anisotropic membrane inclusions such as proteins, protein–lipid complexes, lipid complexes and lipoproteins can be much higher. In this work, we take $\xi_i \approx 5000\ kT\mathrm{nm}^2$ (see Fig. 1) which assumes that the inclusion is a single membrane protein, protein–lipid complex or a cluster of lipid molecules.

If the inclusion perturbs the lipid layer, the lipids in the vicinity of the inclusion change their length and their tilt angle with respect to the normal to the membrane surface [24]. In the case of a rigid inclusion (protein), the excess free energy of the perturbed monolayer per length of the inclusion perimeter ($\Delta F/L$) can be expressed up to the second order in terms of the relative change of the hydrophobic monolayer thickness and the average tilt angle, and their first derivatives [24]. Since we consider the inclusion in a bent monolayer, the excess free energy ($\Delta F/L$) also depends on the radius of the inclusion R_i. Expansion up to the second order leads to a quadratic dependency of ($\Delta F/L$) on R_i. Integration of ($\Delta F/L$) along the whole inclusion perimeter (of length $L = 2\pi R_i$ for a fixed inclusion radius R_i) gives us the expression for the single inclusion (excess) free energy E, which is a cubic function of R_i. Comparison of the thus determined expression for the single inclusion energy E with the corresponding phenomenological expression given in [18] allows us to estimate the interaction constant $\xi_i \approx 3\pi k_{\mathrm{c}} R_i^3/\lambda$ (see [6]), where $\lambda \approx 1$ nm is the typical decay length of the inclusion-induced membrane perturbation [24] and $k_{\mathrm{c}} \approx 20\ kT$ [22]. Estimation of the average inclusion radius (R_i) from the value of the interaction constant $\xi_i \approx 5000\ kT\mathrm{nm}^2$ gives us $R_i \approx (\xi_i \lambda/3\pi k_{\mathrm{c}})^{1/3} \approx 2.9$ nm. In the above estimation of the protein radius R_i, the interactions

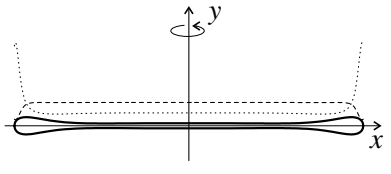

Fig. 1. Calculated equilibrium shape of the Golgi vesicle and the corresponding lateral distributions of the membrane inclusions. Two types of inclusions are considered: inclusions with zero intrinsic principal curvatures ($C_{1\mathrm{m},1} = C_{2\mathrm{m},1} = 0$) that prefer flat central regions (dashed line) and inclusions with one large and one zero intrinsic principal curvature ($C_{1\mathrm{m},2} = 27/R_0$ and $C_{2\mathrm{m},2} = 0$) that prefer highly curved regions on the vesicle rim (dotted line). $N_1 = N_2 = 50 \cdot 8\pi k_{\mathrm{c}}/kT \sim 10^4$. The values of the other parameters are: $V = 0.1 \cdot 4\pi R_0^3/3$ and $\xi_1 = \xi_2 = 0.02 kT R_0^2 \approx 5000\ kT\mathrm{nm}^2$, where $R_0 = \sqrt{A/4\pi} \approx 0.5$ μm. The axisymmetric vesicle shape is parameterized by expression (3).

between the hydrophilic parts of the membrane components were not taken into account. Also the intermolecular van der Waals forces [17] were not considered and therefore $R_i < 3$ nm.

3. Results and discussion

Fig. 1 shows the calculated equilibrium lateral distribution of the membrane inclusions (components) and the corresponding equilibrium shape of the Golgi vesicle obtained by minimization of the membrane free energy (F) for two different types of inclusions ($i = 1, 2$). The lateral distributions of the membrane inclusions are highly non-homogeneous. Inclusions of one type are present mostly in the flat central region of the vesicle, while inclusions of the other type prefer the curved edge of the vesicle. The resemblance with the experimentally observed shapes of real Golgi vesicles can be clearly seen. Note that in the thin central region of the calculated shape, the membranes are separated by a certain distance, as observed in real Golgi vesicles [21].

The results presented in Fig. 1 are in accordance with the most recent theory of transport of carrier vesicles between different cell organelles which assumes that the space variation of the membrane physical properties may determine the location of the membrane bud, or the site of the fusion of the carrier vesicle with the membrane of the organelle [26]. In the case of the Golgi bodies, it was suggested that the COPI proteins are concentrated on the curved bulbous rims of the large flattened Golgi vesicles where budding of the COPI vesicles takes place [26]. On the other hand, the bulbous rims are depleted in sphingomyelin and sterol domains (rafts) that tend to form flat bilayers [13]. Accordingly, COPI vesicles contain significantly reduced levels of sphingomyelin and cholesterol compared with their parental Golgi membranes [2].

A non-homogeneous lateral distribution of membrane components and a different membrane composition of daughter vesicles compared with their parental membrane have been observed experimentally and also indicated theoretically in other membrane systems. For example, in erythrocytes the budding of the daughter exovesicles takes place predominantly on the top of the echinocyte spicula [19], where the local lipid and protein composition of the erythrocyte membrane differs from that of the rest of the membrane since the released vesicles have specific composition [8].

In Golgi bodies, a non-homogeneous lateral distribution has also been indicated for lipid domains. Based on different molecular shapes of lipid molecules (cylindrical, conical and inverted conical) [1,17,20] and phospholipid–cholesterol complexes (inclusions), it was proposed that the proportions of different lipid domains in the flat central part of the Golgi vesicle differ from the proportions of lipid domains on the curved bulbous rim [26].

The characteristic shapes of the flattened Golgi vesicles cannot be explained within the standard bending elasticity models of the bilayer membranes [22]. In such models, the calculated equilibrium shapes of closed lipid bilayer vesicles that most resemble the Golgi vesicles are the torocyte and the codocyte vesicle shapes [9,14]. Such shapes have a thin central region where the membranes on both sides of the vesicle are always in close contact [9,14]. However, as can be seen under the microscope [21], the adjacent membranes in the thin central region of the large flattened Golgi vesicle are always separated by a certain distance, as predicted in Fig. 1.

If the calculated distance between the inner surfaces of opposing membrane in the flat central region of the vesicles is small enough, then the long-range electric double layer repulsion should be taken into account in the minimization procedure. The sugar residues attached to proteins and lipids on the inner surface of Golgi vesicle abolish the short-range attractive van der Waals and the short range attractive or oscillatory hydration forces between the internal surfaces of the vesicle in its flat central region as the estimated range of these forces is not longer than 2 nm [16].

In our theoretical predictions (Fig. 1), the applied value of 5000 $kT\text{nm}^2$ for the interaction constant ξ_i is much larger than the corresponding value for a single isotropic lipid molecule. This means that the candidates for inclusions are membrane proteins, protein–lipid complexes or very small clusters of lipid molecules (nanorafts). Very small rafts may coalesce into larger rafts [13] upon curvature-induced clustering of nanorafts.

In conclusion, we presented a hypothesis that successfully explains the coupling of the observed non-homogeneous lateral distribution of the membrane proteins and lipids, and the specific flattened shape of Golgi vesicles. In spite of the complexity of the real system and the simplifications assumed in our theoretical model of the membrane, the theoretical results presented may add to a better understanding of the mechanisms that maintain the non-homogeneous lateral distribution of membrane components in the membrane of the flattened Golgi vesicles. The predicted non-homogeneous lateral distribution of membrane components is in accordance with recent experimental evidence, indicating that COPI coats and also other membrane constituents are concentrated in certain regions of the Golgi vesicles [13,26].

References

[1] Cullis, P.R. and de Kruijff, B. (1979) Biochim. Biophys. Acta 559, 399–420.
[2] Brugger, B., Sandhoff, R., Wegehingel, S., Gorgas, S., Malsam, J., Helms, J.B., Lehmann, W.D., Nickel, W. and Wieland, F.T. (2000) J. Cell Biol. 151, 507–518.
[3] Duden, R. (2003) Mol. Membr. Biol. 20, 197–207.
[4] Elsner, M., Hashimoto, H. and Nilsson, T. (2003) Mol. Membr. Biol. 20, 221–229.
[5] Fošnarič, M., Nemec, M., Kralj-Iglič, V., Hägerstrand, H., Schara, M. and Iglič, A. (2002) Colloids Surfaces B 26, 243–253.
[6] Fošnarič, M., Kralj-Iglič, V., Bohinc, K., Iglič, A. and May, S. (2003) J. Phys. Chem. B 107, 12519–12526.
[7] Gimsa, J. and Ried, C. (1995) Mol. Membr. Biol. 12, 247–254.
[8] Hägerstrand, H. and Isomaa, B. (1994) Biochim. Biophys. Acta 1190, 409–415.
[9] Deuling, H.J. and Helfrich, W. (1976) J. Phys.-Paris 37, 1345–1355.
[10] Heerklotz, H. (2002) Biophys. J. 83, 2693–2701.
[11] Higgins, M.K. and McMahon, H.T. (2002) Trends Biochem. Sci. 27, 257–263.
[12] Holopainen, J.M., Angelova, M.I. and Kinnuen, P.K.J. (2000) Biophys. J. 78, 830–838.
[13] Holthuis, J.C.M., van Meer, G. and Huitema, K. (2003) Mol. Membr. Biol. 20, 231–241.
[14] Iglič, A., Kralj-Iglič, V., Božič, B., Bobrowska-Hägerstrand, M. and Hägerstrand, H. (2000) Bioelectrochemistry 52, 203–211.
[15] Iglič, A., Hägerstrand, H., Bobrowska-Hägerstrand, M., Arrigler, V. and Kralj-Iglič, V. (2003) Phys. Lett. A 310, 493–497.

[16] Israelachvili, J.N. and Wennerström, H. (1996) Nature 379, 219–225.
[17] Israelachvili, J.N. (1997) Intermolecular and Surface Forces, 2nd ed. Academic Press, London.
[18] Kralj-Iglič, V., Heinrich, V., Svetina, S. and Žekš, B. (1999) Eur. Phys. J. B 10, 5–8.
[19] Kralj-Iglič, V., Iglič, A., Hägerstrand, H. and Peterlin, P. (2000) Phys. Rev. E 61, 4230–4234.
[20] Kralj-Iglič, V., Iglič, A., Gomišček, G., Sevšek, F., Arrigler, V. and Hägerstrand, H. (2002) J. Phys. A 35, 1533–1549.
[21] Lehninger, A.L. (1988) Principles of Biochemisty. Worth, New York.
[22] Lipowsky, R. and Sackmann, E. (1995) Structure and Dynamics of Membranes. Elsevier, Amsterdam.
[23] Marčelja, S. (1974) Biochim. Biophys. Acta 367, 165–176.
[24] May, S. (2002) Langmuir 18, 6356–6364.
[25] Rustom, A., Saffrich, R., Markovič, I., Walther, P. and Gerdes, H.H. (2004) Science 303, 1007–1010.
[26] Sprong, H., van der Sluijs, P. and van Meer, G. (2001) Nat. Cell Biol. 2, 504–513.