Coalescence of phospholipid vesicles mediated by β2GPI – experiment and modelling

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Abstract— **Collective interactions between the giant phospholipid vesicles made of POPC, cardiolipin and cholesterol after the addition of β2GPI may cause the coalescence of membrane buds to the mother cell. Using the discrete elastic model of the vesicle membrane mechanics it was shown that the coalescence of the buds depends on the adhesion strength and rigidity of the biomembrane.**

Keywords— **Beta-2-glycoprotein-I; Apolipoprotein, elastic model, discretization.**

I. Introduction

The serum protein β2GPI is considered to have a multiplicity of physiological roles, among them in the process of blood clot formation. It was found that it affects the metabolism of triacylglycerol-rich lipoproteins, the function of thrombocytes, and the activation of endothelial cells ([1] and references therein) and inhibits the transformation of prothrombin into thrombin [2]. It binds to structures which contain negatively charged phospholipid molecules such as phospholipid vesicles [3,4], thrombocytes [5], thrombocyte-derived microvesicles and apoptotic cells [6], serum lipoproteins [7,8] and mediates cellular recognition of negatively charged phospholipid-exposing microparticles [9-11].

II. Methods

A. β2GPI antibodies

β2GPI (Hyphen BioMed, France) was aliquoted and stored at -70˚C. In all experiments, the final concentration of β2GPI in phosphate buffer saline (PBS) was 100 mg/L, which is approximately half the concentration of physiological β2GPI in normal human plasma (about 200 mg/L) [12,7].

B. Giant phospholipid vesicles

GPVs were prepared at room temperature (23°C) by the electroformation method [13] modified as described in Tomšič et al. [14]. The synthetic lipids cardiolipin (1,1'2,2'-tetraoleoyl cardiolipin), POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), and cholesterol were purchased from Avanti Polar Lipids, Inc. Appropriate volumes of POPC, cardiolipin and cholesterol, all dissolved in a 2:1 chloroform/methanol mixture, were combined in a glass jar and thoroughly mixed. For charged cardiolipin vesicles, POPC, cholesterol and cardiolipin were mixed in the proportion 2:2:1. For neutral POPC vesicles, POPC and cholesterol were mixed in proportion 4:1. Cholesterol was added to POPC to increase the longevity of vesicles. 10 μL of lipid mixture was applied to platinum electrodes. The solvent was allowed to evaporate in a low vacuum for 2 hours. The coated electrodes were placed in the electroformation chamber which was then filled with 3 mL of 0.2 M sucrose solution. An AC electric current with an amplitude of 5 V and a frequency of 10 Hz was applied to the electrodes for 2 hours, which was followed by 2.5 V and 5 Hz for 15 minutes, 2.5 V and 2.5 Hz for 15 minutes and finally 1 V and 1 Hz for 15 minutes. The content was rinsed out of the electroformation chamber with 5 mL of 0.2 M glucose and stored in a plastic test tube. The vesicles were left for sedimentation under gravity for one day at 4 °C. 200 to 400 μL of the sediment was collected from the bottom of the tube and used for a series of experiments. Before placing the vesicles into the observation chamber, the sample was gently mixed.

C. Mathematical model

The mechanical behavior of the vesicle biomembrane is described using an elastic continuum theory where the bindings between the building blocks of the membrane (phospholipids) are represented as the elastic springs. For the sake of simplicity, two dimensional problem was considered. Energetically optimal deformed shape is computed via minimization of the elastic energy of a discrete linear model of lipids interaction.

The model of coalescence of phospholipid vesicles is based on the equilibrium between the two energy contributions: the adhesion energy (*Ea*) due to the contact, and the

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bending and stretching elastic energy (*Ee*) of the membrane due to deformation. The adhesion energy was taken to be depended on the contact surface area linearly (Ea = γ A). As the deformation is prescribed by the shape of the contact area, the elastic energy is linearly dependent on an elastic constant, *K*. Therefore, the total energy of the system is computed as the energy difference between the elastic and the adhesion energy normalized to the elastic constant (*K*). Coefficient Γ describes dependence of the adhesion energy on the dimensionless contact area.

It was considered that the mother and bud vesicles have a form of sphere before adhesion with the radius of 1μm and 0.2 μm, respectively. The volume of the bud vesicle was taken as fixed. The thickness of the membrane was 5 nm.

III. Results and discussion

β2GPI caused coalescence of cardiolipin-containing as well as of POPC vesicles. Adhesion to the bottom of the observation chamber occurred simultaneously. Formation of sticky complexes was also observed in the sample containing both kinds of vesicles. This indicates that β2GPI mediates the interaction between the charged –charged, charged - neutral and neutral - neutral pairs of membranes.

If β2GPI was present in the solution, the bud (Fig.1A-C) had coalesced with the mother vesicle before it could detach from it (Fig.1D-F).

Fig. 2 presents dependence of the ratio between the energy and the elastic constant on the magnitude of the dimensionless coalescence area for various coefficient of adhesion (Γ). Increasing the area of the contact, the elastic energy is increasing while the adhesion energy decreases. Equilibrium of the system is characterized by the minimum in the total energy. It is obvious from Fig. 2 that the area of contact in equilibrium state depends on the adhesion coefficient: i.e. is zero for no adhesion (Γ=0) and large for strong adhesion (Γ=14000 nm^3). The calculated shapes of equilibrium states correspond with the shapes observed in experiment.

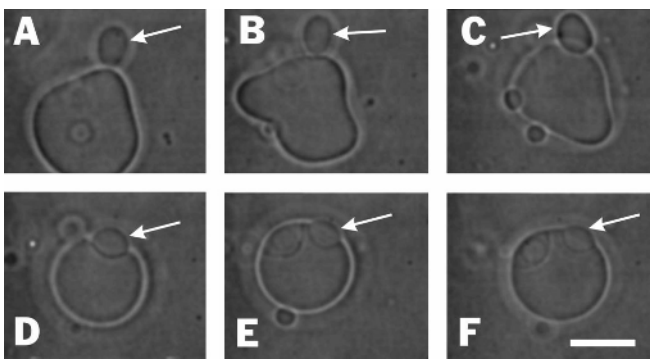

Fig. 1: The effect of β2GPI dissolved in phosphate buffer saline (PBS) on a budding vesicle (A-F). The bud (marked by a white arrow) coalesced with the mother vesicle and remained attached to it. Bar denotes 10μm.

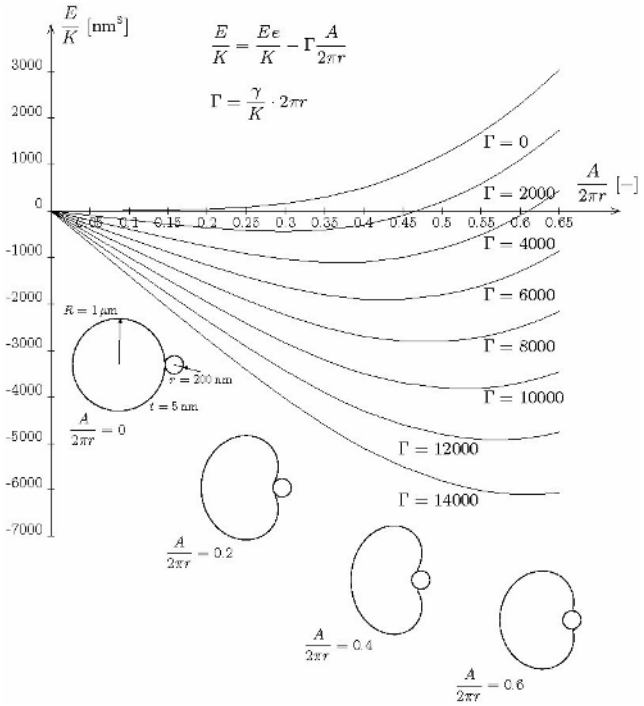

Fig. 2: Dependence between the dimensionless contact area (*A)* and total energy *E* normalized to the elastic constant (*K*). The equilibrium shapes of mother vesicle for various contact areas are shown.

IV. Conclusions

The presented model provides a simplified analysis of the problem neglecting the liquidity of the bilayer and reducing the problem to two dimensions. However, good agreement between the experimental measurements and the model simulations show that the phospholipid vesicle shapes during the coalescence of vesicles mediated by β2GPI may be explained as an interplay between the deformation and contact energy. To describe this phenomenon in details, the mathematical model should be developed further.

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References

1. Bevers EM, Janssen MO, Comfurius P, Balasubramanian K, Schroit AJ, Zwaal RF, Willems GM. (2005) Quantitative determination of the binding of β2-glycoprotein I and prothrombin to phosphatidylserine-exposing blood platelets. Biochem J. 386:271–279.
2. Nimpf J, Bevers EM, Bomans PH, Till U, Wurm H, Kostner GM, Zwaal RF. (1986) Prothrombinase activity of human platelets is inhibited by beta 2-glycoprotein-I. Biochim Biophys Acta. 884:142-149.
3. Wurm H. (1984) β2-glycoprotein I (apolipoprotein H) interactions with phospholipid vesicles. Int J Biochem. 16:511-515.
4. Chonn A, Semple SC, Cullis PR. (1995) β2-glycoprotein I is a major protein associated with very rapidly cleared liposomes in vivo, suggesting significant role in the immune clearance of non-self particles. J Biol Chem. 270:25845-25849.
5. Schousboe I. (1980) Binding of beta 2-glycoprotein I to platelets: effect of adenylate cyclase activity. Thromb Res. 19:225-237.
6. Price BE, Rauch J, Shia MA, Walsh MT, Lieberthal W, Gilligan HM, O'Laughlin T, Koh JS, Levine JS. (1996) Anti-phospholipid autoantibodies bind to apoptotic, but not viable, thymocytes in a beta 2-glycoprotein I-dependent manner. J Immunol. 157:2201-2208.
7. Polz E, Kostner GM. (1979) The binding of beta 2-glycoprotein-I to human serum lipoproteins: distribution among density fractions. FEBS Lett. 102:183-186.
8. Kobayashi K, Kishi M, Atsumi T, Bertolaccini ML, Makino H, Sakairi N, Yamamoto I, Yasuda T, Khamashta MA, Hughes GR, Koike T, Voelker DR, Matsuura E. (2003) Circulating oxidized LDL forms complexes with β_2-glycoprotein I: implication as an atherogenic autoantigen. J Lipid Res. 44:716-726.
9. Balasubramanian K, Chandra J, Schroit AJ. (1997) Immune clearance of phosphatidylserine- expressing cells by phagocytes. The role of beta2-glycoprotein I in macrophage recognition. J Biol Chem. 272:31113-31117.
10. Moestrup SK, Schousboe I, Jacobsen C, Leheste JR, Christensen EI, Willnow TE. (1998) β_2-glycoprotein-I (apolipoprotein H) and β_2-glycoprotein-I-phospholipid complex harbor a recognition site for the endocytic receptor megalin. J Clin Invest. 102:902-909.
11. Thiagarajan P, Le A, Benedict CR. (1999) β_2-glycoprotein I promotes the binding of anionic phospholipid vesicles by macrophages. Arterioscler Thromb Vasc Biol. 19:2807-2811.
12. 27.McNally T, Mackie IJ, Isenberg DA, Machin SJ. (1993) Immunoelectrophoresis and ELISA techniques for assay of plasma beta 2 glycoprotein-I and the influence of plasma lipids. Thromb Res. 72:275-286.
13. Angelova MI, Soléau S, Méléard P, Faucon JF, Bothorel P. (1992) Preparation of giant vesicles by external AC electric fields. Kinetics and applications. Progr Colloid Polym Sci. 89:127–131.
14. Tomšič N, Babnik B, *Lombardo* D, Mavčič B, Kandušer M, Iglič A, Kralj-Iglič V. (2005) Shape and size of giant unilamellar phospholipid vesicles containing cardiolipin. J Chem Inf Model. 45:1676-1679.

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