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Encapsulation of small spherical liposome into larger flaccid liposome induced by human plasma proteins

J.I. Pavlič[a,b], T. Mareš[c], J. Bešter[b], V. Janša[b], M. Daniel[c] and A. Iglič[b]*

[a]*University College of Health Care, University of Ljubljana, Ljubljana, Slovenia;* [b]*Faculty of Electrical Engineering, Ljubljana, Slovenia;* [c]*Laboratory of Biomechanics, Faculty of Mechanical Engineering, Czech Technical University in Prague, Prague, Czech Republic*

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We show that human plasma can induce the encapsulation of small spherical liposomes into larger flaccid liposomes. To explain the observed phenomena, it is proposed that the orientational ordering of charged plasma proteins induces attractive interaction between two like-charged liposome surfaces in close contact. It is observed that the encapsulation of the spherical liposome is possible only if the membrane of the target liposome is flexible enough to adapt its shape to the shape of the spherical liposome. In the theoretical model, the shapes of the two agglutinated liposomes are determined by minimisation of the sum of the adhesion energy and the membrane elastic energy. In the simulations, the membrane of liposomes is considered as an elastic structure and discretised via the finite element method using spring elements. It is shown that the observed agglutination of liposomes and encapsulation of smaller spherical liposomes into larger flaccid liposomes may be explained as a competition between the membrane deformation energy and the membrane adhesion energy.

Keywords: adhesion energy; encapsulation; lipid membranes; modelling; plasma proteins

1. Introduction

Liposomes are promising drug carriers as they may perform a target-oriented release of drugs (Cevc 1995). Therefore the study of mechanisms of interaction of liposomes with the membrane target cell is of special importance. The liposome–cell membrane interactions depend among others on the elastic properties of the membrane bilayer (Kralj-Iglič et al. 1993; Sackmann 1994; Boulbitch 1998), on the physical properties of membrane proteins (Božič et al. 1915; Kralj-Iglič et al. 1996; Kralj-Iglič et al. 1999; McMahon and Gallop 2005), on the cytoskeleton and membrane skeleton (Hägerstand et al. 1999) and on the interactions between membrane surfaces which are strongly influenced by composition and the electric charge of the two interacting membranes (Israelachvili 1991; Cevc 1995). The variation of the lateral composition of the membrane (Devaux and Morris 2004) may additionally facilitate the adhesion between the liposome and the membrane of target cells (Urbanija et al. 2007). Strong adhesion between liposome and cellular membrane may result in a total or partial encapsulation of the liposome (Hägerstrand et al. 1999; Boulbitch 2002). Stimulated by our previous experimental results indicating the importance of the protein-mediated attractive interaction between lipid membranes (Urbanija et al. 2007) we studied in this work the influence of human plasma on the interaction between like-charged liposomes prepared by the modified electro formation method. For comparison between experiments and theory, we modelled the agglutination of two liposomes based on the minimisation of the sum of the membrane adhesion energy and the membrane elastic energy.

2. Materials and methods

Two millilitres of venous blood from a healthy blood donor who gave written consent was collected into a vacutube containing trisodium citrate and processed within 15 min. Following the centrifugation of blood (1500*g*, 15 min, 20°C, SIGMA 3K18 Centrifuge, Sigma, St Louis, MO, USA), plasma was collected and used immediately. Liposomes (giant phospholipid vesicles) were prepared by the modified electroformation method, originally proposed by Angelova et al. (1992). The 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. (Alabaster, AL, USA). Cardiolipin molecules have a net negative charge at physiological conditions. Appropriate volumes of POPC, cardiolipin and cholesterol, all dissolved in 2:1 chloroform/methanol mixture, were combined in a glass jar and thoroughly mixed. POPC, cholesterol and cardiolipin were mixed in the proportion 2:2:1. Cholesterol was added to POPC to increase the

*Corresponding author. Email: ales.iglic@fe.uni-lj.si

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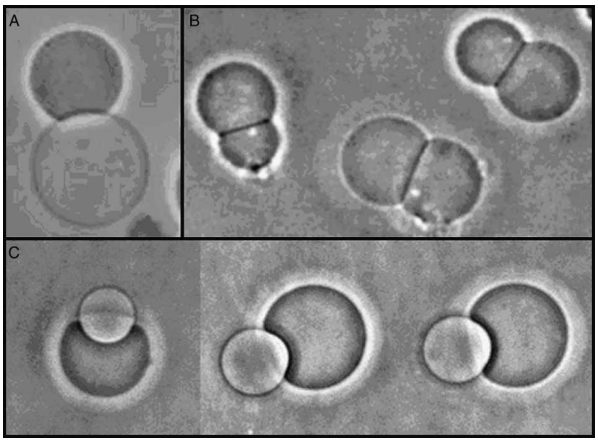

Figure 1. Phase contrast micrographs showing agglutionation of different pairs of negatively charged liposomes after the addition of human plasma. (A) weak adhesion of smaller flaccid liposome to larger spherical liposome, (B) agglutination of two flaccid lipsomes, (C) strong adhesion of smaller spherical liposome to larger flaccid liposome (partial encapsulation).

longevity of vesicles. Twenty microlitres of the lipid mixture was applied to each of the two platinum electrodes shaped as rods (approximate length 4 cm and diameter 1 mm). The electrodes were left in a low vacuum for 2 h for solvent to evaporate. The lipid-coated electrodes were thereafter placed into a microcentrifuge tube filled with 2 ml of 200 mM sucrose solution to form an electroformation chamber. An AC electric voltage with an amplitude of 5 V and a frequency of 10 Hz was applied to the electrodes for 2 h, which was followed by 2.5 V and 5 Hz for 15 min, 2.5 V and 2.5 Hz for 15 min and finally 1 V and 1 Hz for 15 min. After the electroformation, 600 μl of sucrose solution containing liposomes and 1 ml of 200 mM glucose solution were pipetted in three 2-ml plastic microcentrifuge tubes which were sealed with parafilm band to prevent entrance of air and to protect the solution from microorganisms. The liposomes were left for sedimentation under gravity in a low vacuum at room temperature for one day. The liposomes were observed by an inverted microscope Zeiss Axiovert with phase contrast optics. All experiments were performed at room temperature.

3. Experimental results

Figure 1 shows three different types of agglutinated liposomes 30 min after addition of human plasma. Before the addition of plasma, the liposomes were separated while after the addition of the plasma they agglutinated. The size of the contact area between the agglutinated lipsomes in general depends on the strength of the adhesion energy (adhesion forces), on the areas of both liposomes and on the relative volumes of liposomes (see also Evans 1995; Boulbitch 2002). Here the relative volume of the liposome (v) is defined as: $v = V/(4\pi R^3/3)$, where V is the volume of the liposome, $R = (A_0/4\pi)^{1/2}$, and A_0 is the area of the liposome (see also Urbanija et al. 2007). For maximal possible values of the relative volume $v = 1$, the liposome has a spherical shape. In the case when the relative volumes of two agglutinated liposomes approach the value 1, the size of the contact area approaches zero. The necessary condition for the non-zero value of the size of the contact area between the two lipsomes is that at least one of the two interacting liposomes has its relative volume smaller than one.

If the relative volumes of two interacting liposomes are smaller than 1, the shape of the contact area between two agglutinated liposomes is usually planar (Figure 1(B)). If one liposome is spherical with the relative volume close to one, the other liposome with relative volume $v < 1$ can be adhered to the membrane of spherical liposome in a way that the contact area has a positive mean curvature (Figure 1(A)). This case simulates the attachment of the liposome to the cell membrane which cannot be strongly deformed indicating that the membrane encapsulation of the liposome is possible only in the case when the membrane of the target cell is flexible enough to adapt its shape to the more or less spherical shape of the liposome. This is shown in Figure 1(C) where the relative volume of the smaller liposome is close to 1, while the relative volume of the larger liposome is small enough to allow partial encapsulation of the small liposome into the larger flaccid liposome.

The results presented in Figure 1 show that human plasma can induce an attractive interaction between membranes even when they are like-charged. The recent accompanying theoretical and modelling studies on protein-mediated interactions between like-charged surfaces indicate that plasma proteins can induce attractive interaction between membranes due to positional and orientational distribution of protein molecules with spatially distributed charge within a single protein molecule (Bohinc et al. 2004).

The mutual attraction between like-charged membrane surfaces may be partially the result of various other factors (Evans 1995) such as bridging by the mediating molecules bound to both surfaces (Bombeli et al. 1998), attractive electrostatic forces in the two interacting electric double layers generated by charged surfaces in contact with an electrolyte solution (Carnie and Torrie 1984), van der Waals interaction (Evans 1995) and suppression of membrane fluctuations (Helfrich 1995).

4. Theoretical consideration and conclusions

The above described experimental results, showing the possibility of encapsulation of spherical liposome (Figure 1) are theoretically discussed by using a simple mathematical

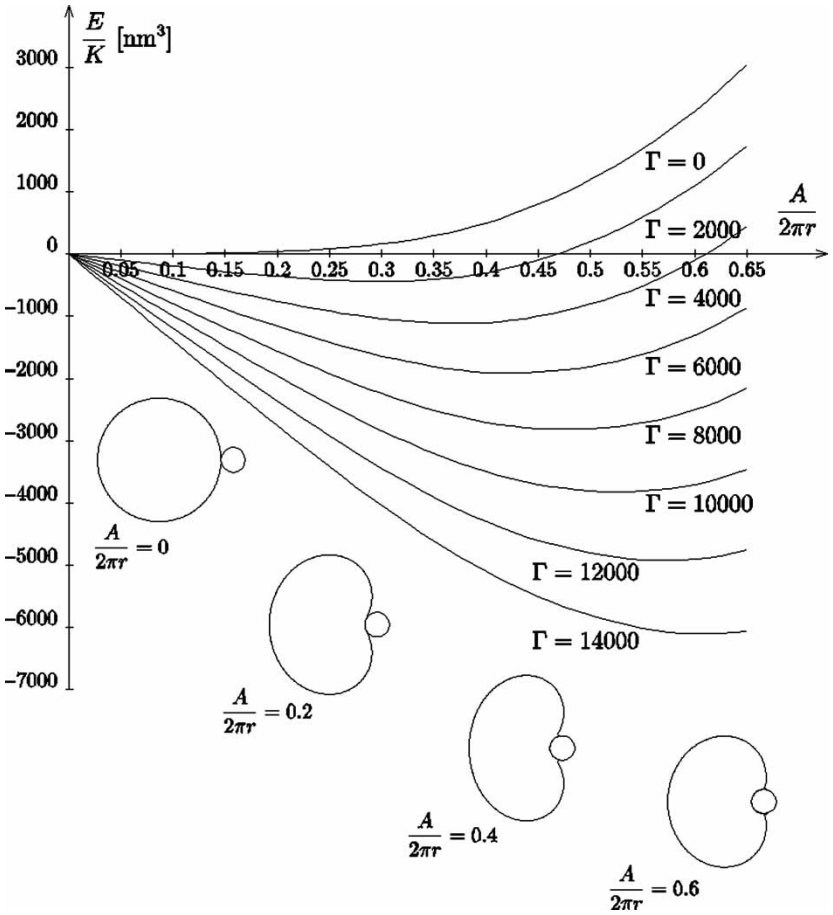

Figure 2. Normalised total energy of the system (E/K) as a function of the normalised size of the contact line ($A/2\pi r$) calculated for different values of the normalised adhesion constant $\Gamma = (\gamma 2\pi r)/K$. The equilibrium shapes of liposomes are also shown for four values of ($A/2\pi r$) and $\Gamma = 0$, 2000, 6000, 14,000, respectively. The thickness of the membrane is 5 nm, the radius of small lipsome is 200 nm and the radius of large liposome is 1 μm.

model. Namely, we calculated the equilibrium shape of two adhered liposomes by minimisation of the total of energy of the system. The mechanical properties of the phospholipid membrane of the liposomes is described within continuum elastic theory.

For the sake of simplicity, only the two-dimensional problem was considered. Energetically optimal deformed shape and size of the area of contact between adhered liposomes is computed via minimisation of the total energy of the system where a discrete linear model of lipid interaction is adopted.

In the model, the total energy of the system (E) is assumed to be composed of adhesion energy and the elastic energy of the membranes of adhered liposomes (E_{el}) due to bending and stretching of the membrane:

$$E = E_{\text{el}} - \gamma A, \tag{1}$$

where the adhesion $E_{\text{ad}} = -\gamma A$ energy was taken to be linearly dependent on the size of the contact region (A), while the elastic energy is assumed to be linearly dependent on the elastic constant K. The larger (non-spherical) liposome is modelled as an elastic structure and discretised via the finite element method using spring elements (Szabo and Babuska 1991; Zienkiewicz et al. 2005) to describe the interactions between the building blocks of phospholipid membrane. For the given size of the contact region, the elastic energy of the membranes of adhered lipsomes is calculated using standard finite element procedure.

Figure 2 shows the total energy of two agglutinated liposomes as a function of the normalised size of the contact region ($A/2\pi r$) calculated for different values of the normalised adhesion constant $\Gamma = (\gamma 2\pi r)/K$. It can be seen in Figure 2 that by increasing the region of contact between the two liposomes, the total energy of the liposomes first decreases due to a decrease of the adhesion energy which prevails over the increase of the elastic (bending) energy of the large liposome. For large enough A, the total energy of liposomes E starts to increase due to strong increase of the elastic (bending) energy E_{el}. Equilibrium configuration of the system is characterised

by the minimum of the total energy E. It is seen in Figure 2 that the size of the contact region in equilibrium state strongly depends on the adhesion coefficient Γ. For zero value of normalised adhesion constant ($\Gamma = 0$) the equilibrium size of the contact region approaches zero, while in the limit of strong adhesion (i.e. large Γ) the smaller spherical liposome can be completely encapsulated by the membrane of the larger liposome.

To conclude, the presented theoretical model provides a simplified analysis of the problem neglecting the liquid behaviour of the lipid bilayer and reducing the problem to two dimensions. However, a good qualitative agreement between the experimental observations and the model simulations shows that the shapes of two interacting liposomes may be explained as a competition between the deformation of the membrane and the energy of adhesion.

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