Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioelectrochemistry 63 (2004) 183–187
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Shape transformation of giant phospholipid vesicles at high concentrations of $C_{12}E_8$

B. Mavčič, B. Babnik, A. Iglič*, M. Kandušer, T. Slivnik, V. Kralj-Iglič

Faculty of Electrical Engineering, University of Ljubljana, Tržaška 25, SI-1000 Ljubljana, Slovenia

Received 23 June 2003; received in revised form 28 August 2003; accepted 19 September 2003

Abstract

Giant unilamellar phospholipid vesicles were prepared by the method of electroformation from 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC). We studied the influence of different concentrations of the surfactant octaethyleneglycol dodecylether ($C_{12}E_8$) on the spontaneous shape transformations of POPC vesicles at room temperature. In accordance with previous results, we observed that low concentration of $C_{12}E_8$ increased the speed of the characteristic vesicle shape transformation, starting from the initial shape with thin tubular protrusion, through beaded protrusion where the number of beads gradually decreased, to final spherical shapes with invagination, whereby the average mean curvature of the vesicle membrane monotonously decreased. In contrast, higher concentration of $C_{12}E_8$ initially induced the shape transformation in the "opposite direction": in the protrusion, the number of beads gradually increased and eventually a tube was formed whereby the average mean curvature of the vesicle membrane gradually increased. However, at a certain point, an abrupt shape change took place to yield the vesicle with invagination. In this transition, the average mean curvature of the vesicle membrane discontinuously decreased. After this transition, the vesicle began to shrink and finally disappeared. We discuss possible mechanisms involved in the observed transformations.

Keywords: Giant phospholipid vesicles; Nanostructures; Electroformation; $C_{12}E_8$ Surfactant; Micelles

1. Introduction

Phospholipid giant unilamellar vesicles, produced by electroformation, have been extensively studied in the past [1–3], and the research was focused mainly on the changing shape and budding transition states of the vesicles [1,4]. The studied phospholipids included 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC), 1-stearoyl-OPC (SOPC) and dioleylphosphatidylcholine (DOPC). Studies on influence of temperature [1] and various agents (specific enzymes, La^{3+}, Gd^{3+}, alcohol, surfactants) have shown transformations of the original vesicle into different shapes. In particular, addition of nonionic surfactant octaethyleneglycol dodecylether ($C_{12}E_8$) at low concentrations increased the speed of vesicle transformation with long, thin, tubular protrusions; the transition of the protrusion from tube to a chain of vesicles and the final shape with invaginations [5]. The aim of our work was to observe the transition states and shape transformations of POPC giant unilamellar vesicles after addition of higher $C_{12}E_8$ concentrations than in the previous studies [5] and to compare the results with transformation without added surfactant in the original solution [3–5].

2. Experimental

Phospholipid giant unilamellar vesicles were prepared at room temperature by electroformation, as described extensively in the previous publications [3–6]. We used the 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) phopholipid, produced by Avanti Polar Lipids. POPC was dissolved in 9:1 chloroform/methanol solution and applied to two platinum electrodes. The solvent was allowed to evaporate in vacuum exicator for 2 h. The coated electrodes were then placed in the electroformation chamber, filled with 4 ml of 0.2 M sucrose solution, and an AC

* Corresponding author. Tel.: +386-1-4768-825; fax: +386-1-4264-630.
E-mail address: ales.iglic@fe.uni-lj.si (A. Iglič).

1567-5394/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2003.09.022

electric field was applied according to the previously used protocol [5]. The content of the chamber was put into a test tube and 4 ml of 0.2 M glucose solution was added. Afterwards, on the cover glass the observation chamber was created by silicon grease in the shape of two rectangular compartments connected through a 3-mm-wide gap, in order to enable slow diffusion of surfactant into the adjacent compartment because direct addition of surfactant to the vesicle solution resulted in very fast solubilization and disappearance of vesicles before we were even able to observe them with microscope. One compartment was filled with 30 μl of the vesicle solution, and in case of added surfactant, the other compartment was additionally filled with 15 μl of 18.6 or 1.86 mM nonionic surfactant $C_{12}E_8$. The vesicles were observed at room temperature with ZEISS 200 Axiovert inverted phase microscope at 1000× immersion magnification.

3. Results

3.1. POPC vesicle transformation without $C_{12}E_8$ or with $C_{12}E_8$ at low concentration

Following the electroformation process, the vesicles made of phospholipid only initially adopted a spherical appearance. After 30 min, the first thin, tubular protrusions became visible under the microscope. The long, thin, tubular protrusions began to transform into beads, and the beads then gradually reduced in number to yield a fluctuating globular vesicle. The transformation continued by forming a spherical vesicle with invagination, whereby the average mean curvature of the vesicle membrane was continuously decreasing. A scheme of such membrane transformation based on mathematical modelling is shown in Fig. 1 [5]. The whole transformation took several hours to complete and it was consistent with previous observations in similar conditions [3–5]. Addition of the 1.86 mM surfactant to the compartment of the observation chamber adjacent to the vesicle solution increased the speed of the transformation process [5]; however, it did not significantly change its course.

3.2. POPC vesicle transformation after addition of $C_{12}E_8$ at high concentration

Immediately after the 18.6 mM surfactant solution was added to the compartment of the observation chamber adjacent to the vesicles solution, the vesicles appeared spherical and no protrusions were visible. After approximately 30 min, the vesicles acquired prolate bean-like shape with undulating membrane and in some cases the shape was reversibly interchanging into the shape of vesicle with one bead, and vice versa (Fig. 2A–N). In time, the one-bead shape progressed further, the beads increased in number to form a 6-bead-long protrusion (Fig. 2O–Y). Evidently, the

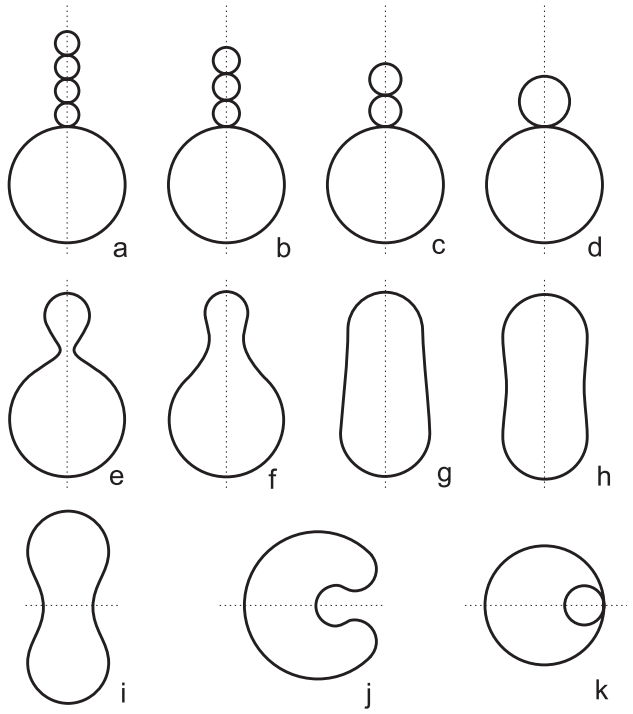

Fig. 1. Scheme of theoretically predicted axisymmetric equilibrium vesicle shapes corresponding to minimal isotropic local bending energy at constant vesicle volume and area for different values of normalized average mean curvature (h): 1.62 (a), 1.53 (b), 1.43 (c), 1.30 (d), 1.25 (e), 1.17 (f), 1.086 (g), 1.085 (h), 1.02 (i), 0.77 (j) and 0.66 (k). Relative cell volume $v = 0.85$. The broken lines show the axes of rotational symmetry (adapted from Ref. [5]).

average mean curvature of the vesicle membrane was gradually increasing. At a certain point, the vesicle with beaded protrusion abruptly changed shape: the necks between the beads opened and the beads fused together to form a tubular structure connected to the mother globule by a thick neck (Fig. 3A). The tubular structure then exhibited vigorous fluctuations for a short time (Fig. 3B–F), followed by an instant formation of invagination (Fig. 3G). The average mean curvature of the vesicle membrane discontinuously and substantially decreased during the formation of the invagination (Fig. 3). In due course, the mother vesicle with the invagination began to shrink and eventually disappeared altogether, as depicted in Fig. 3I–N. The presented pattern of vesicle shape transformation at high surfactant concentration has been observed in more than 10 independent experiments.

4. Discussion

Observations of the giant POPC vesicles in the solution without the surfactant and with low 1.86 mM surfactant concentration correspond to the previously observed transformation pattern—long, thin protrusions; thick tubes; chain of beads; mother vesicle with invagination, whereby the average mean curvature of the vesicle membrane is contin-

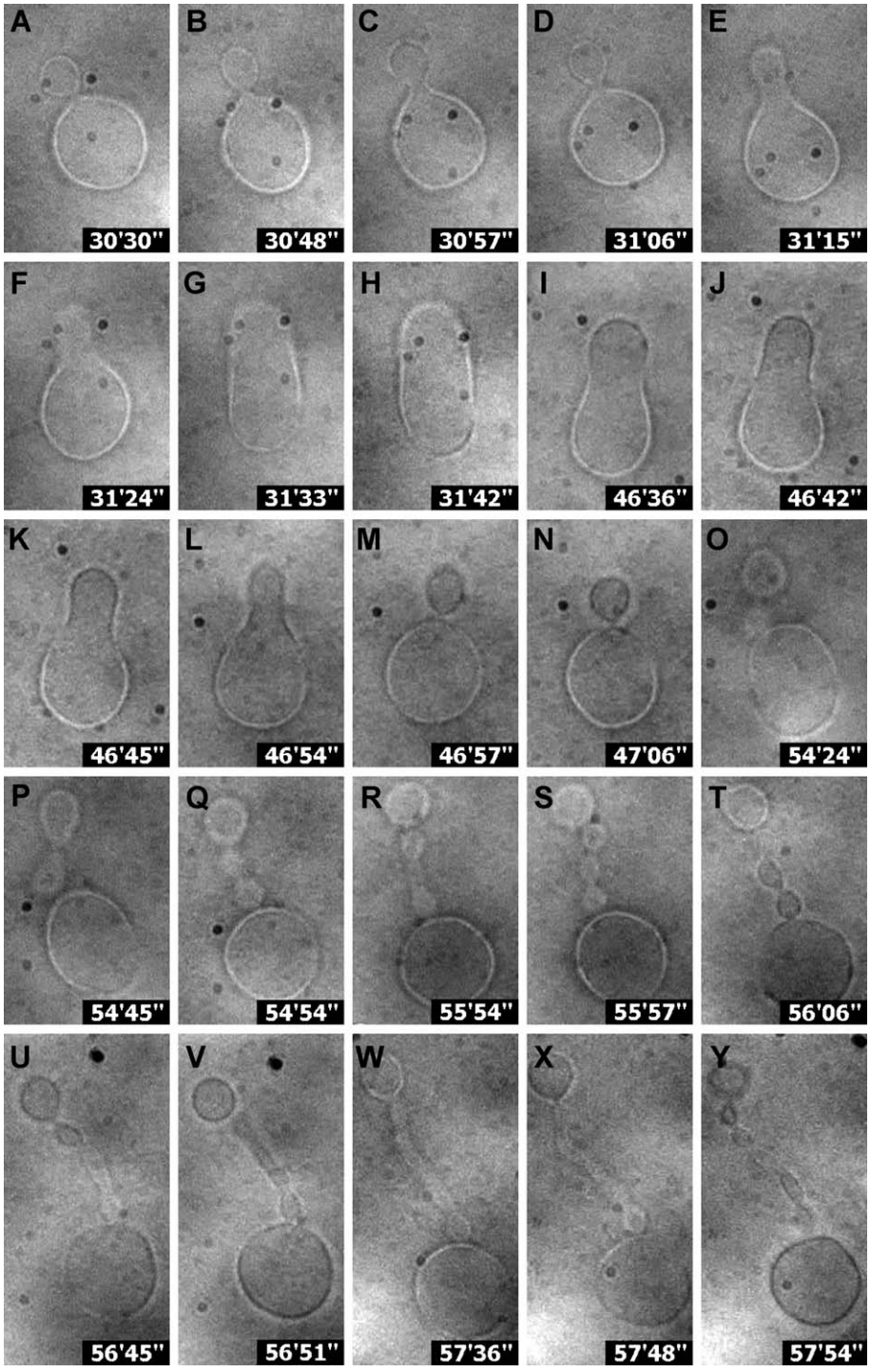

Fig. 2. The initially observed transition phase of a giant POPC vesicle with one bead to a bean-like shape (A–H) and back to the one-bead form (I–N), the protrusion then gradually grows to six beads (Y). The recorded times in minutes (′) and seconds (″) are measured from the time point of $C_{12}E_8$ surfactant addition. Width of a single image corresponds to 20 μm.

uously decreasing [3–5]. However, the transformation pattern of giant POPC vesicles after the addition of 18.6 mM $C_{12}E_8$ surfactant solution in the adjacent compartment significantly differs from the ones previously considered. The fluctuating globular vesicle developed a bead-like protrusion where the number of the beads increased, reflecting an increase of the average mean curvature. Then, at a certain point, the transformation pattern was abruptly reversed and the average mean curvature discontinuously decreased.

Explanation of the observed phenomena is at present beyond our limits of understanding. However, we can point to some mechanisms that could be involved in the observed process. Theoretical analyses of membrane transformations

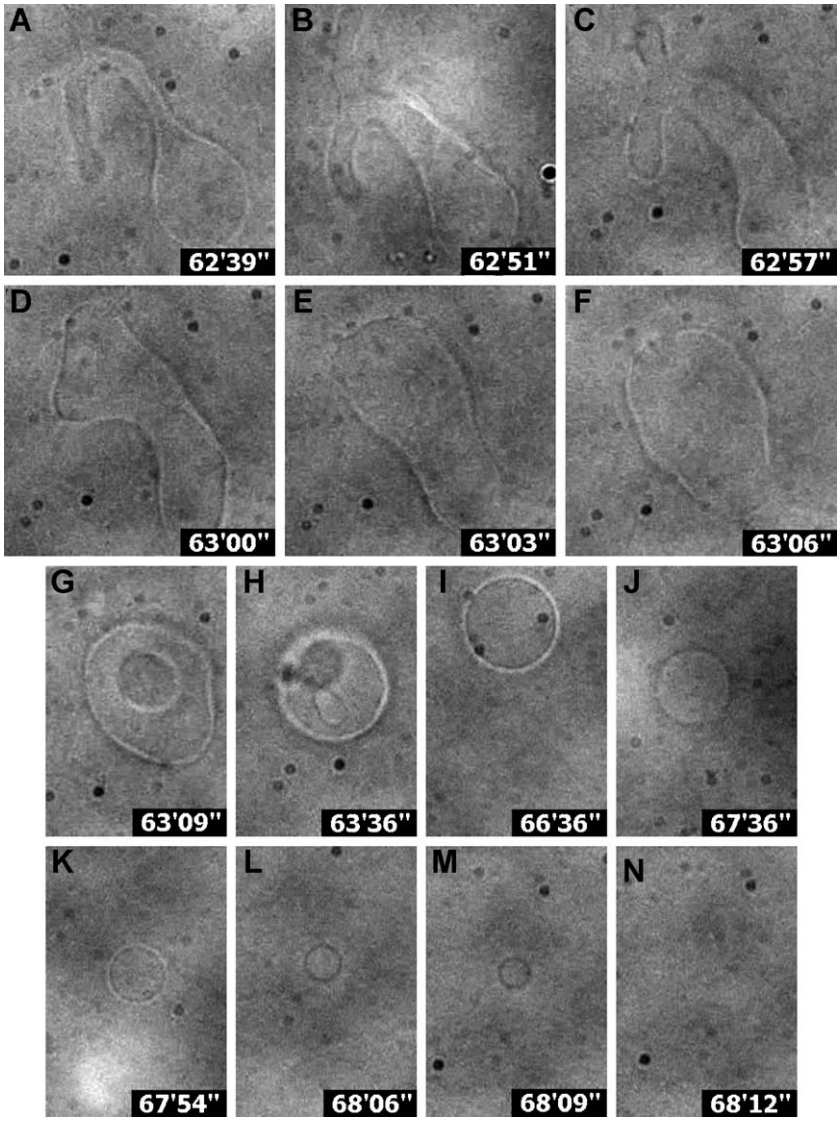

Fig. 3. The final transition phase of the giant POPC vesicle from Fig. 2. The beaded protrusion in Fig. 2Y has now acquired tubular form and it rapidly fuses together to a prolate bean-like form, which than transforms to a vesicle with an invagination (A–G). Within the next 5 min, the vesicle gradually shrinks and disappears altogether (H–N). The recorded times in minutes (′) and seconds (″) are measured from the time point of $C_{12}E_8$ surfactant addition. Width of a single image corresponds to 30 and 20 μm for square and rectangular images, respectively.

have shown that the average mean curvature, which changes during the process, is proportional to the area difference between the outer and the inner monolayer of the bilayer lipid membrane [2,5]. The areas of both membrane layers may change during the process because the surfactant molecules are likely to associate with the membrane. It is possible that the surfactant molecules intercalate differently into the two membrane layers. As the intercalated detergent molecule has a finite area per molecule, this would cause a change of the difference between the areas of the two membrane layers. Besides, the membrane-intercalated molecules may strongly influence and rearrange the adjacent phospholipid molecules, thereby disrupting the quasiequilibrium state of the membrane and resulting in the change of the area difference (i.e., the average mean curvature) of the vesicle. In addition, formation of mixed micelles could take place within the phospholipid bilayer as well as in the solution. Formation of the surfactant aggregates within the membrane may strongly influence the local membrane curvature. It was suggested that $C_{12}E_8$ stabilizes membrane pores in cells [7] by concentrating on the toroidally shaped rims of the pores. These experiments suggest that the intrinsic shape of $C_{12}E_8$-induced membrane inclusions favors toroidal geometry and such phenomena could also take place in phospholipid vesicles. Mechanisms involving the phospholipid molecules only may also be important such as the phospholipid flip-flop, the drag of the phospholipid molecules from the solution by the glass walls of the observation chamber and chemical modification of the phospholipid [3].

In the presence of high surfactant concentration, we have observed spatial and temporal lags between transformation

processes of individual vesicles within the same observation chamber. In addition, we believe that sudden discontinuous changes from increasing to decreasing average mean curvature may be the consequence of spatial and temporal changes in the concentration of the diffusing surfactant.

In conclusion, our results show that at higher concentrations of surfactant the shape transformation of the phospholipid vesicle does not follow the continuous change of the average mean curvature caused by intercalation of the surfactant into the membrane. It is indicated that discontinuous aggregation processes involving surfactant and phospholipid take place and strongly influence the shape of the phospholipid vesicle.

References

[1] J. Käs, E. Sackmann, Shape transitions and shape stability of giant phospholipid vesicles in pure water induced by area-to-volume changes, Biophys. J. 60 (1991) 825–844.

[2] F. Nomura, M. Nagata, T. Inaba, H. Hiramatsu, H. Hotani, K. Takiguchi, Capabilities of liposomes for topological transformation, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 2340–2345.

[3] V. Kralj-Iglič, G. Gomišček, J. Majhenc, V. Arrigler, S. Svetina, Myelin-like protrusions of giant phospholipid vesicles prepared by electroformation, Colloids Surf. A, (2001) 315–318.

[4] V. Kralj-Iglič, A. Iglič, G. Gomišček, F. Sevšek, V. Arrigler, H. Hägerstrand, Microtubes and nanotubes of a phospholipid bilayer membrane, J. Phys. A, Math. Gen. 35 (2002) 1533–1549.

[5] B. Babnik, D. Miklavčič, M. Kandušer, H. Hägerstrand, V. Kralj-Iglič, A. Iglič, Shape transformation and burst of giant POPC unilamellar liposomes modulated by nonionic detergent $C_{12}E_8$, Chem. Phys. Lipids 125 (2003) 123–128.

[6] M.I. Angelova, S. Soleau, Ph. Meleard, J.F. Faucon, P. Bothorel, Preparation of giant vesicles by external AC electric field: kinetics and application, Prog. Colloid & Polym. Sci. 89 (1992) 127–131.

[7] M. Kandušer, M. Fošnarič, M. Šentjurc, V. Kralj-Iglič, A. Iglič, K. Bialkowska, B. Isomaa, H. Hägerstrand, Effect of surfactant polyoxyethyleneglycol ($C_{12}E_8$) on electroporation of cell line DC3F, Colloids Surf. A 214 (2003) 205–217.