J|A|C|S
COMMUNICATIONS

Published on Web 09/14/2004

Membrane-Bound Protein in Giant Vesicles: Induced Contraction and Growth

Victor A. Seredyuk and Fredric M. Menger*

Department of Chemistry, Emory University, Atlanta, Georgia 30322

Received June 10, 2004; E-mail: menger@emory.edu

Giant vesicles (GVs) have provided chemists and biophysicists with a valuable model for cell membranes.[1] Being cell-sized, GVs introduce no annoying curvature issues as do the more widely studied submicroscopic vesicles[2] with diameters in the 100 nm range. GVs also undergo a variety of "cytomimetic" events, including fission, fusion, budding, birthing, and endocytosis.[3] Yet to date there has been an obvious difference between cell membranes and their GV models: Cell membranes commonly contain 18−85% protein,[4] a component omitted in most GV systems. Thus, to more faithfully simulate cell membranes, we have begun to examine GVs with a high protein content, and we report our initial results herein. As will be demonstrated, the presence and depletion of protein from GV bilayers can have important consequences with regard to vesicle morphology.

The protein in question here is called "zein". Zein, isolated from corn endosperm, has been used commercially in the preparation of slow-release tablets, plastics, adhesives, ceramics, fibers, and coatings.[5] It has a globular structure, a molecular weight of 25 kDa, an isoelectric point of 7.2, and a high (20%) leucine content. Zein is water-insoluble but soluble in many organic solvents, including ethanol and glycol ethers. Surfactants can solubilize zein in water.[6] Zein's stability and hydrophobicity make it an ideal subject for studying membrane-bound proteins in GV bilayers.

In a typical GV preparation, a film was deposited on the two parallel Pt wires of an electroformation cell[7] by coating them with a mixture of phospholipids (neutral POPC and anionic POPG)[8] and zein (92:5:3 mol %) in CH_2Cl_2/CH_3OH (9:1) and removing the solvent with a N_2 stream. An initial alternating current (10 Hz, 100 mV) was applied after filling the cell with water or buffer. The voltage was increased to 1.0 V as the frequency was progressively decreased to 3.0 Hz over 3−4 h. At cessation of the current, giant vesicles (20−100 μm in diameter and presumably unilamellar) had been formed on the wires. These were observed by phase-contrast microscopy using a Nikon Diaphot-TMD inverted microscope in tandem with an Optronics DEI-750TD Peltier-cooled 3-CCD color camera. Data were processed with the aide of Image Pro Plus 4.0.

Assuming a GV with a 60-μm diameter, an area per lipid of 9.6 nm^2, and a 5.0-nm diameter zein embedded at its equator, then a 2 mol % zein in the bilayer occupied nearly half the vesicle surface. Thus, the classical fluid mosaic model of Singer and Nicolson[9] has been more realistically simulated.

When anionic, zein-free POPC/POPG giant vesicles (95:5 mol % in 2 mL of water, pH = 6.7) were exposed to 40 μL of 10 mM sodium dodecyl sulfate (SDS), no surfactant-induced GV alterations were observed. If, however, the GV bilayer possessed 2 mol % zein, then the SDS caused a dramatic size reduction over the course of half an hour (Figure 1). The percent decrease in diameter is linearly related to the zein content of the GVs up to 3 mol % (Figure 2). Diluted SDS solutions (150 μL, 1.0 mM) had no effect on the zein-bearing GVs. Nor did a simple inorganic salt, Na_2SO_4 (100 μL, 20 mM). Cholesterol exhibited a protective effect upon the GVs. Thus, GVs composed of POPC/POPG/cholesterol/zein (80:8:10:2) did not respond to SDS additions.

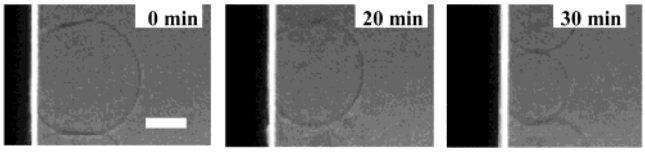

Figure 1. Shrinkage of giant vesicle composed of POPC/POPG/zein (92:5:3 mol %, 2.0 mL, pH = 6.7) induced by 40 μL, 10 mM SDS. The final SDS concentration in the electroformation cell (0.2 mM) is submicellar. The scale bar represents 25 μm.

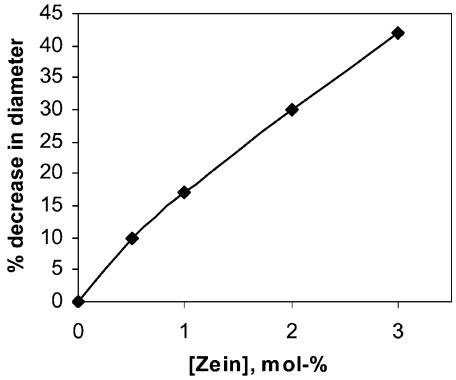

Figure 2. Change in the diameter of giant vesicles POPC/POPG/zein in the presence of SDS (10 mM, 40 μL) after 1 h as a function of the zein in the bilayer. Composition of POPC/POPG/zein (from left to right) = (95:5), (94.5:5:0.5), (94:5:1), (93:5:2), and (92:5:3), respectively. The line is a visual guide.

Surfactant interactions with zein have been examined just recently.[6] It was shown that at 2−4 mM SDS the surfactant is incorporated into the globular zein to form micelle-like microdomains that help solubilize the protein. Similarly, the SDS may remove zein from the GV membranes via protein/surfactant complexes. Remarkably, the giant vesicles can heal themselves and maintain their vesicular morphology despite the gross damage that must occur when the protein departs.

pH is a critical factor in determining protein migration from membrane to bulk phase. At pH = 6.7 and 4.2 (below the 7.2 isoelectric point), the protein was removed by SDS. At pH = 8.7, where zein is negatively charged, anionic surfactant induced no GV contraction. On the other hand, 20 μL of 50 mM dodecyltrimethylammonium bromide removed zein at pH = 8.7 but not at pH = 4.2 or 6.7. Clearly, both hydrophobic and electrostatic forces between zein and surfactant are at play here.

Membrane-incorporated protein can also be instrumental in GV growth. Such a growth was observed (Figure 3) when GVs were exposed to small unilamellar vesicles (SUVs), about 100 nm in diameter. Thus, the following components were mixed in the order cited: (a) GVs of POPC, POPG, and zein (93:5:2 mol % in 2 mL of water, pH = 6.8), (b) SUVs of DDAB[8] (100 μL, 5 mg/mL), and

10.1021/ja040151s CCC: $27.50

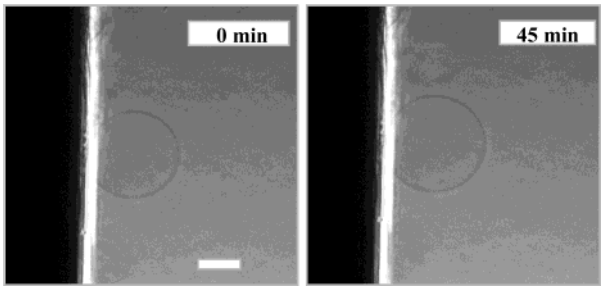

Figure 3. Growth of zein-containing giant vesicles consisting of POPC/POPG/zein (93:5:2 mol %) after addition of small DDAB vesicles (100 μL, 5 mg/mL) followed by addition of SDS (50 μL, 10 mM). The scale bar represents 25 μm.

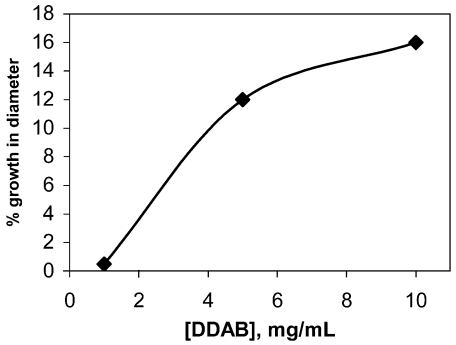

Figure 4. Diameter of anionic giant vesicles composed of POPC/POPG/zein (93:5:2 mol %) as a function of the DDAB concentration constituting the small cationic unilamellar vesicles. Growth is initiated by addition of 50 μL, 10 mM SDS.

(c) SDS (50 μL, 10 mM) now acting as a growth initiator. In this experiment, the GVs were negatively charged owing to the 5% POPG, whereas the SUVs were composed of positively charged DDAB. The GV growth in Figure 3 can be reasonably attributed to GV/SUV fusion, a fusion that was not observed in the absence of zein. Nor did fusion occur with the positive SUVs if the GVs were neutral (no POPG) or if the SUVs themselves were positive (5% DDAB in place of POPG). Furthermore, no growth of negative GVs was seen when the SUVs were neutral or negative (composed of POPC or POPC/POPG). GV growth as a function of the DDAB content is given in Figure 4.

Thus, the combined elements required for GV growth are 4-fold: negative GV, positive SUV, protein, and SDS (Figure 5). A likely mechanism involves an electrostatically driven adsorption of the SUVs onto the much larger GVs. Previous work has demonstrated a strong binding affinity between vesicles of opposite charge.[10] Now the high-curvature SUVs have been no doubt destabilized by the SDS (SUVs are actually destroyed by higher concentrations of surfactant).[11] These weakened SUVs then fuse with the GVs. A clue as to why zein is necessary for fusion comes from a recent publication by Kogan et al.[12] This group worked with zein's N-terminal repetitive domain, $(VHLPPP)_8$, which is thought to be necessary for vesicular binding. It was found that interaction between the peptide and small vesicles increases the membrane

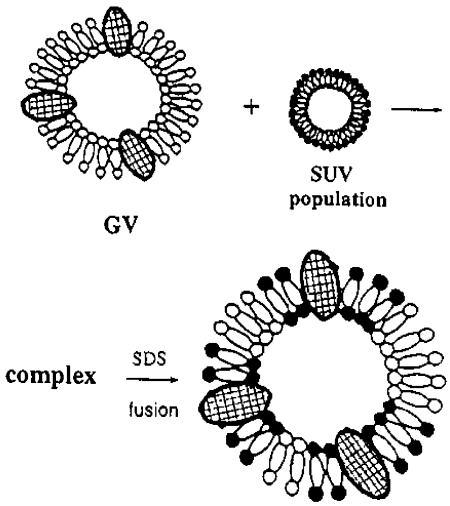

Figure 5. Schematic depiction of GV growth. Diagram is not to scale (with the GV being, in reality, 500× larger than the SUVs). Zein is indicated by shaded ovals. GV lipid pairs are represented by open "dumbbells", and SUV lipid pairs are represented by solid "dumbbells". After fusion, rapid lateral diffusion would randomize the GV- and SUV-derived lipids (not shown).

permeability owing, ostensibly, to a change in lipid organization. GV/SUV fusion might therefore arise from membrane defects especially at the periphery of the protein molecules. Accordingly, GV growth occurs only in the presence of zein-created "fusion hotspots". One is reminded here of virus "fusion proteins" that reconfigure host membranes prior to viral entry.[13]

Electrostatics seemingly serves a dish of SDS-tenderized small vesicles to the giant vesicles, but the feast does not begin unless protein is around to ring the dinner bell. Afterward, to continue the metaphor, the giant vesicles loosen their belts.

Acknowledgment. This work was supported by the National Institutes of Health.

References

(1) *Giant Vesicles*; Luisi, P. L., Walde, P.; Eds.; Wiley: New York, 2000.
(2) Lasic, D. D. *Liposome: From Physics to Applications*; Elsevier: Amsterdam, 1993.
(3) Menger, F. M.; Gabrielson, K. D. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2091.
(4) Stein, W. D. *The Movement of Molecules across Cell Membranes*; Academic Press: New York, 1967; p 13.
(5) Lawton, J. W. *Cereal Chem.* **2002**, *79*, 1.
(6) Deo, N.; Jockusch, S.; Turro, N. J.; Somasundaran, P. *Langmuir* **2003**, *19*, 5083.
(7) Menger, F. M.; Angelova, M. I. *Acc. Chem. Res.* **1998**, *31*, 789.
(8) POPC = 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPG = 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (Avanti); DDAB = didodecyldimethylammonium bromide (Fluka).
(9) Singer, S. J.; Nicolson, G. L. *Science* **1972**, *175*, 723.
(10) Menger, F. M.; Seredyuk, V. A.; Yaroslavov, A. A. *Angew. Chem., Int. Ed.* **2002**, *41*, 1350.
(11) Kadi, M.; Hansson, P.; Almgren, M. *J. Phys. Chem. B* **2004**, *108*, 7344.
(12) Kogan, M. J.; Lopez, O.; Cocera, M.; Lopez-Iglesias, C.; De la Maza, A.; Giralt, E. *Biopolymers* **2004**, *73*, 258.
(13) Chernomordik, L. V.; Kozlov, M. M. *Annu. Rev. Biochem.* **2003**, *72*, 75.

JA040151S