J|A|C|S
COMMUNICATIONS

Published on Web 09/13/2002

Depth-Profiling with Giant Vesicle Membranes

Fredric M. Menger,* Jason S. Keiper, and Kevin L. Caran
Department of Chemistry, Emory University, Atlanta, Georgia 30322

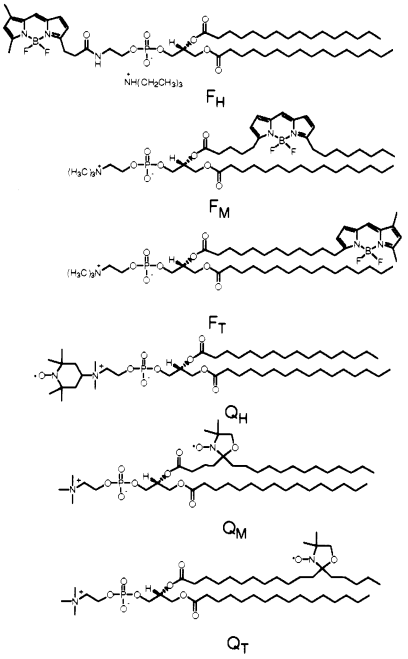
Received June 14, 2002

In recent years, the giant vesicle has become a favorite subject of the popular scientific press with the corresponding commentary often being accompanied by eye-catching photographs.[1] Perhaps chemists enjoy a brief diversion from graphs, tables, and spectra while gazing upon the remarkable "cytomimetic" processes in which giant vesicles engage (for example, fusion, fission, budding, endocytosis, birthing, etc.).[2] Giant vesicles' visual appeal should not, however, obscure the fact that they have provided, with the aid of the optical microscope, unique information on the nature of biomembranes.[3−6] Although submicroscopic vesicles (20−500 nm) have comprised the vast majority of model biomembrane work in the past, giant vesicles (1−100 μm) offer an advantage expressed in the cliché that "seeing is believing". A versatile technology has already developed around the giant vesicle including electroformation,[7] laser immobilization,[8] micromanipulation,[9] microinjection,[10] and two-photon fluorescence methods.[11] As will be shown herein, we have now focused on the use of epi-fluourescence microscopy in locating the binding sites of membrane-bound adsorbants. Thus, "How deep does a foreign guest penetrate a giant vesicle membrane?" is the central question addressed in this paper.

Scheme l shows three lipids (F_H, F_M, and F_T) labeled at the head, middle, and terminus with the fluorescent BODIPY unit.[12] These were individually incorporated, at 1 mol %, into giant vesicles composed primarily of phospholipid SOPC (1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine). Also embedded within the giant vesicle membranes (at 1−15 mol %) was one of three potent fluorescence quenchers: lipids that had been spin-labeled at the head, middle, or near the terminus (Q_H, Q_M, and Q_T in Scheme 1).[13] The resulting combinations of nine quenching efficiencies, measured quantitatively by epi-fluorescence microscopy, provided the distances at which the fluorophores reside from the center of the giant vesicle bilayer. Although certainly not a new strategy,[14] it has never been applied to giant vesicle systems whose curvature is far more planar and cell-like than that found in the common submicroscopic vesicles made by sonication, extrusion, or vortexing. Unnaturally high curvature in such small vesicles affects their lipid packing[15] and, by this means, might alter adsorption sites in comparison with those found in the cell membrane and in its most true-to-life model—the giant vesicle.

A few technical details essential to the success of the experiments should be mentioned. Giant vesicles (believed to be unilamellar)[3] were made by the electroformation method developed by Angelova et al.[3,7] Thus, a Pt wire coated with a mixture of SOPC, fluorophore, and quencher was hydrated with deionized water at 22−24 °C and subsequently subjected to an increasing voltage (0.1−3.5 V) and decreasing frequency (10−0.5 Hz) over the course of 2−5 h to produce 10−60 μm wire-bound giant vesicles. Epi-fluourescence microscopy[16] was carried out with a Nikon Diaphot-TMD inverted microscope, an OSRAM HBO 100 W lamp, a NikonB-2A filter combination cube (λ_{ex} = 450−490 nm), and an Optronics DEI-750TD Peltier-cooled 3-CCD color camera in tandem with an Optronics color monitor.

Scheme 1. Fluorophores and Quenchers



Vesicles were examined individually, and great care was taken to handle them all equally. An electroformed vesicle was removed from the Pt wire (using a micropipet and a Narishige micromanipulator attached to a Nikon PLT-188 pico-injector to control suction) and then released near the bottom of the chamber in a region isolated from other vesicles. After the camera was set for 0.25−1.0 s exposure times, the shutter from the microscope's lamp housing was opened manually for about l s (irradiation times of greater than 2 s leading to photobleaching). At that point, the image was captured (using the camera's freeze-frame feature) and downloaded to the PC where fluorescence intensities were analyzed by "line-profiling" (Image Pro-Plus).[17] As seen in Figure 1, increasing amounts of spin-label quencher (0−15 mol %) diminish the fluorescence intensities of the BODIPY units.

Two formats are provided in Figure l: (a) a replica of what is actually seen under the microscope and (b) a so-called "surface plot" that gives a more three-dimensional representation of intensities. These data can be transformed into depth information via use of the "parallax equation" (eq 1) as developed by London et al.[18,19] In this equation, Z_{cF} is the average distance of the fluorophore from the bilayer center, C is the concentration of quencher in molecules/Å^2 (assuming 65 Å^2/SOPC molecule), and F_1 and F_2 are the relative fluorescence intensities of the vesicle bilayers in the presence of a shallow and deep quencher, respectively. L_{21} is the distance between the shallow and deep quenchers in Å, and L_{C1} is the distance of the shallow quencher from the bilayer center in Å. Because L_{C1}

* To whom correspondence should be addressed. E-mail: menger@emory.edu.

10.1021/ja020838h CCC: $22.00

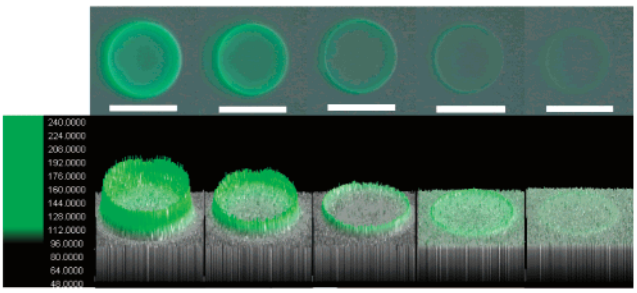


Figure 1. Composite intensity surface plots of SOPC/F_M/Q_H representative vesicles. From left to right: 0%, 1%, 5%, 10%, and 15% Q_H. Bars = 25 μm.

Table 1. Fluorescence Intensity Data for 1 mol % F_M in SOPC Giant Vesicles

	vesicles profiled	diameter average (μm)	diameter std dev (μm)	intensity average	intensity std dev	relative fluorescence intensity
no quencher	20	28.5	3.6	106.1	12.3	1
Q_H						
1 mol %	20	28.2	4.2	80.3	11.8	0.76
5 mol %	10	29.8	2.7	34.7	3.5	0.33
10 mol %	10	24.8	3.6	22.3	2.3	0.21
15 mol %	10	31.6	6.2	17.1	2.1	0.16
Q_M						
1 mol %	20	28.2	3.5	73.4	10.2	0.69
5 mol %	10	30.4	5.9	51.3	5.63	0.48
10 mol %	10	34.7	6.9	30.8	3.2	0.29
15 mol %	10	25.6	2.8	24.5	1.2	0.23
Q_T						
1 mol %	20	27.4	2.8	63.0	6.4	0.59
5 mol %	10	26.1	2.4	38.0	5.7	0.36
10 mol %	10	25.9	2.8	26.2	1.2	0.25
15 mol %	10	26.2	3.6	21.5	2.1	0.20

Table 2. Fluorophore Distances from Bilayer Centers[a]

fluorophore	distance (Å)
F_H	17.7
F_M	20.6
F_T	19.2

[a] Estimated uncertainty of ±1.5 Å.

and L_{21} are known from ESR data,[20] and because F_1, F_2, and C are obtained experimentally, the fluorophore distance from the center can be calculated.

$$Z_{cF} = \frac{\left[\left(\frac{1}{-\pi C}\ln\frac{F_1}{F_2}\right) - L_{21}^{\,2}\right]}{2L_{21}} + L_{C1} \qquad (1)$$

Ten to twenty giant vesicles were profiled and averaged for each of the three fluorophores (F_H, F_M, and F_T) using four membrane concentrations of either Q_H, Q_M, or Q_T. Raw data from three of nine such experiments using F_M are given in Table 1. Substituting relative fluorescence intensities at 15 mol % quencher from Table 1 (and from similar tables for the other two fluorophores) into the parallax equation yielded the estimated fluorophore depths given in Table 2.

Uncertainty in the parallax method as applied to giant vesicles arises from at least four sources: (a) a less than total labeling in one or more of the quencher lipids;[21] (b) a possible variability in the nitroxyl residence sites; (c) multilamellar membranes and subsequent complications from interlayer adsorption; and (d) data that are not sufficiently precise to define small but finite populations in a nonunimodal distribution of fluorophore residence sites. Past discussions in the literature[18−22] allow us to regard all but the last of these problems as minor. A weak dependence of fluorescence intensity on vesicle size has been reported,[23] and for this reason we selected giant vesicles with diameters as similar to each other as possible (Table l).

It is clear from this study that giant vesicles do indeed lend themselves to quantitative analysis by the parallax method. The resulting data in Table 2 show that the calculated depths for the giant vesicles are within the experimental error of those obtained from submicroscopic vesicles.[21] In other words, despite the known effect of a high radius of curvature upon chain disorder in small vesicles,[24−26] the binding sites (at least with BODIPY) for small and giant vesicles are identical.

All BODIPY fluorophores, even the one at the terminus of the lipid chain, prefer to reside at the membrane surface, fully 17−20 Å distant from the bilayer center. Clearly, the lipid chains bearing the BODIPY units in F_M and F_T must "loop" to bring the units to the vesicle periphery. (Because the SOPC is in the liquid crystalline phase at our experimental temperature, small molecules can rapidly attain a positional equilibrium within the bilayers.[27]) Since looping likely disrupts the bilayer structure, forces (of which we have little understanding) must more than compensate for the resulting lipid disorder. The well-known affinity of quaternary ammonium groups for π-systems,[28] and the sizable interfacial area offered by a vesicle to an adsorbant, are two likely factors favoring surface adsorption. The tendency of even modestly polar molecules (or sections of molecules) to gravitate to the membrane surface has numerous practical ramifications. For example, in past attempts to construct artificial channels,[29] one must be alert to the possibility that the channel compounds bind to the membrane surface, disrupt the packing, and enhance transport processes without actually forming discrete channels.

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

References

(1) *Chem. Eng. News* **1997**, *75(45)*, 19. Wilson, E. *Chem. Eng. News* **1999**, *77(12)*, 7. *Chem. Eng. News* **1999**, *77(20)*, 51. Brennan, M. *Chem. Eng. News* **1999**, *77(32)*, 7.
(2) Menger, F. M.; Gabrielson, K. D. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2091.
(3) Menger, F. M.; Angelova, M. I. *Acc. Chem. Res.* **1998**, *31*, 789.
(4) Luisi, P. L., Walde, P., Eds. *Giant Vesicles*; John Wiley and Sons: Chichester, 2000.
(5) Seifert, U. *Adv. Phys.* **1997**, *46*, 13.
(6) Döbereiner, H.-G.; Evans, E.; Kraus, M.; Seifert, U.; Wortis, M. *Phys. Rev. E* **1997**, *55*, 4458.
(7) Angelova, M. I.; Dimitrov, D. S. *Faraday Discuss. Chem. Soc.* **1986**, *81*, 303.
(8) Dietrich, C.; Angelova, M.; Pouligny, B. *J. Phys. II France* **1997**, *7*, 1651.
(9) Menger, F. M.; Keiper, J. S. *Adv. Mater.* **1998**, *10*, 888.
(10) Holopainen, J. M.; Angelova, M. I.; Kinnunen, P. K. J. *Biophys. J.* **2000**, *78*, 830.
(11) Bagatolli, L. A.; Gratton, E. *Biophys. J.* **2000**, *78*, 290.
(12) Johnson, I. D.; Kang, H. C.; Haugland, R. P. *Anal. Biochem.* **1992**, *198*, 228.
(13) Fluorophores were purchased from Molecular Probes (Eugene, OR), and quenchers were purchased from Avanti Polar Lipids (Pelham, AL).
(14) Palmer, L. R.; Merrill, A. R. *J. Biol. Chem.* **1994**, *269*, 4187.
(15) Sheetz, M. P.; Chan, S. I. *Biochemistry* **1972**, *11*, 4573.
(16) Rost, F. W. D. *Quantitative Fluorescence Microscopy*; Cambridge University Press: Cambridge, 1991.
(17) Servuss, R.-M.; Boroske, E. *Chem. Phys. Lipids* **1980**, *27*, 57.
(18) Chattopadhyay, A.; London, E. *Biochemistry* **1987**, *26*, 39.
(19) Kaiser, R. D.; London, E. *Biochemistry* **1998**, *37*, 8180.
(20) Abrams, F. S.; London, E. *Biochemistry* **1993**, *32*, 10826.
(21) Kaiser, R. D.; London, E. *Biochim. Biophys. Acta* **1998**, *1375*, 13.
(22) Mathivet, L.; Cribier, S.; Devaux, P. F. *Biophys. J.* **1996**, *70*, 1112.
(23) Akashi, K.-I.; Miyata, H.; Itoh, H.; Kinosita, K. *Biophys. J.* **1996**, *71*, 3242.
(24) Lentz, B. R.; Barenholz, Y.; Thompson, T. E. *Biochemistry* **1976**, *15*, 4521.
(25) Parmar, Y. I.; Wassall, S. R.; Cushley, R. J. *J. Am. Chem* Soc. **1984**, *106*, 2434.
(26) Wu, W.-G.; Dowd, S. R.; Simplaceanu, V.; Peng, Z.-Y.; Ho, C. *Biochemistry* **1985**, *24*, 7153.
(27) Träuble, H.; Sackmann, E. *J. Am. Chem. Soc.* **1972**, *94*, 4499.
(28) Mecozzi, S.; West, A. P., Jr.; Dougherty, D. A. *J. Am. Chem. Soc.* **1996**, *118*, 2307.
(29) Gokel, G. W.; Murillo, O. *Acc. Chem. Res.* **1996**, *29*, 425.

JA020838H