Communications to the Editor

Light Microscopic Investigations of the Autocatalytic Self-Reproduction of Giant Vesicles

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In an attempt to create self-reproducing vesicles,[1] we have recently described experiments in which the hydrolysis of oleic acid esters[2a] or oleic anhydride[2b−d] occurs within the boundary of oleic acid/oleate vesicles, leading to an increase of the number of vesicles. It is worthwhile to recall the experimental configuration of the previous work, since basically it is the same as here. We start with a biphasic system comprising a water suspension of preformed oleic acid vesicles overlayed with a droplet of neat, water-insoluble oleic anhydride. Under these conditions, the anhydride hydrolysis reaction is catalyzed by the vesicle bilayer. The rate of hydrolysis within the first 3 h is increased, for example, by a factor of 30 in the presence of 20 mM oleic acid/oleate vesicles in comparison with the hydrolysis in buffer alone.[2b] The reaction products (oleic acid and oleate) are incorporated into the vesicles, and this in turn leads to a growth of the vesicle size and number in an exponential autocatalytic time process. Since this process takes place within the boundary of the parent vesicles, it can be defined as an autopoietic[3] self-reproduction.[1]

Although we have shown that the hydrolysis of oleic anhydride is catalyzed by oleic acid/oleate vesicles, the mechanisms underlying vesicle self-reproduction could not be visualized directly, as we were generally dealing with vesicles of 100−200 nm diameters. Thus the experimental evidence for an increase in number and/or size of vesicles during the hydrolysis reaction resided mainly in electron microscopy. Since the preparation of samples for electron microscopy is lengthy and often cumbersome, we consider here giant vesicles which have the advantage that the reproduction process and other possible shape and size transformations can be observed directly under the light microscope, without particular sample preparation. The preparation and characterization of giant vesicles has been described by several authors[4−10] for a variety of different surfactant molecules. In the present work, we wish to directly evidence the self-reproduction of oleic acid/oleate giant vesicles (OGVs).

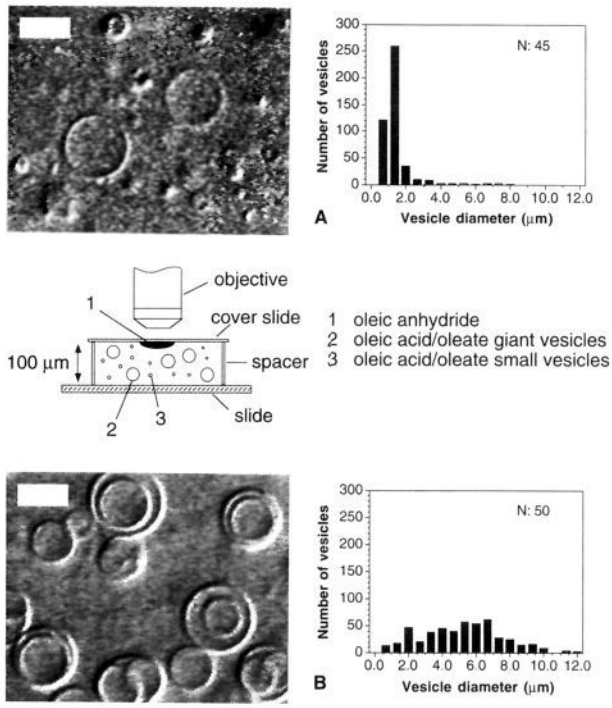

Figure 1. Visualization by video-enhanced light microscopy (differential interference contrast) of changes in size and number of oleic acid/oleate giant vesicles (OGVs) during intralamellar oleic anhydride hydrolysis. (A) OGVs in the absence of oleic anhydride at 25 °C. (B) OGVs during oleic anhydride hydrolysis (6 h after addition of the anhydride at 25 °C). The white scale bar represents 5 μm. Ten micrographs were taken at different places in the solution and analyzed for OGV size (>0.5 μm) and number as shown on the right-hand side. In the case of vesicles containing another vesicle in the interior ("inclusion vesicles"), only the outer vesicle is counted. The numbers of vesicles per micrograph (*N*) are also given. Inset: Scheme of the experimental setup under the microscope, showing a cross-sectional view of the microscopic slide.

Spontaneously formed OGVs (50 mM lipid) are prepared by dispersing a thin film of sodium oleate in 0.2 M bicine (*N,N*-bis[2-hydroxyethyl]glycine) buffer, pH 8.5, as described before.[2b,11] This vesicle dispersion is further diluted 10 times with buffer and analyzed by light microscopy (Zeiss Axioplan). Among smaller vesicles, very large, spherical vesicles with diameters up to 10−20 μm are present.[12] These vesicles remain stable for several hours without undergoing shape transformations, even during a heating and cooling cycle control experiment.

The situation changes completely upon addition of a drop of oleic anhydride (3 μL = 0.5 μmol) to the vesicle suspension (200 μL = 1 μmol of lipid) under the microscope. A series of

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(1) In contrast to "self-replication", which is limited to linear information sequences based on template-assisted recognition principles, "self-reproduction" is used as a more general term, in particular for the case of micelles and vesicles. The specification "autopoietic self-reproduction" is relative to a case in which the reproduction process is due to a reaction occurring within the boundary of the structure itself. Luisi, P. L. In *Self-Production of Supramolecular Structures*; Fleischaker, G. R., Colonna, S., Luisi, P. L., Eds.; Kluwer Academic: Dordrecht, 1994; pp 196−197.

(2) (a) Vonmont-Bachmann, P. A.; Walde, P.; Luisi, P. L. *J. Liposome Res.* **1994**, *4*, 1135−1158. (b) Walde, P.; Wick, R.; Fresta, M.; Mangone, A.; Luisi, P. L. *J. Am. Chem. Soc.* **1994**, *116*, 11649−11654. (c) Walde, P.; Goto, A.; Monnard, P.-A.; Wessicken, M.; Luisi, P. L. *J. Am. Chem. Soc.* **1994**, *116*, 7541−7547. (d) Oberholzer, T.; Wick, R.; Luisi, P. L.; Biebricher, C. K. *Biochem. Biophys. Res. Commun.*, in press.

(3) (a) Varela, F.; Maturana, H.; Uribe, R. *BioSystems* **1974**, *5*, 187−196. (b) Maturana, H.; Varela, F. *Autopoiesis and Cognition: The Realization of the Living*; D. Reidel: Boston, 1980.

(4) Reeves, J. P.; Dowben, R. M. *J. Cell. Physiol.* **1969**, *73*, 49−60.

(5) Mueller, P.; Chien, T. F.; Rudy, B. *Biophys. J.* **1983**, *44*, 375−381.

(6) (a) Sackmann, E.; Duwe, H.-P.; Engelhardt, H. *Faraday Discuss. Chem. Soc.* **1986**, *81*, 281−290. (b) Käs, J.; Sackmann, E. *Biophys. J.* **1991**, *60*, 825−844.

(7) Ringsdorf, H.; Schlarb, B.; Venzmer, J. *Angew. Chem.* **1988**, *100*, 117−162.

(8) (a) Angelova, M. I.; Dimitrov, D. S. *Prog. Colloid Polym. Sci.* **1988**, *76*, 59−67. (b) Angelova, M. I.; Soléau, S.; Méléard, Ph.; Faucon, J. F.; Bothorel, P. *Prog. Colloid Polym. Sci.* **1992**, *89*, 127−131.

(9) Farge, E.; Devaux, P. F. *Biophys. J.* **1992**, *61*, 347−357.

(10) (a) Menger, F. M.; Balachander, N. J. *J. Am. Chem. Soc.* **1992**, *114*, 5862−5863. (b) Menger, F. M.; Gabrielson, K. *J. Am. Chem. Soc.* **1994**, *116*, 1567−1568.

(11) The pH is kept to a value which is close to the pK of the acid in the bilayer, the requirement for linear fatty acids to build vesicles: (a) Gebicki, J. M.; Hicks, M. *Nature* **1973**, *243*, 232−234. (b) Hargreaves, W. R.; Deamer, D. W. *Biochemistry* **1978**, *17*, 3759−3768. (c) Haines, T. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 160−164. (d) Cistola, D. P.; Hamilton, J. A.; Jackson, D.; Small, D. M. *Biochemistry* **1988**, *27*, 1881−1888.

(12) Remarkably, the largest oleic acid/oleate vesicles which we have observed by light microscopy have a diameter of about 80 μm.

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rather fascinating transformations can be observed by light microscopy (Figure 1B, point 2), due to the catalytic hydrolysis of the anhydride taking place at the boundary of the vesicles. As shown schematically in the inset of Figure 1, the droplet is in direct contact with the vesicle suspension. The anhydride molecules are possibly transferred from the anhydride droplet into the bilayer of the vesicles through collision processes in which the vesicles are in transient contact with the anhydride droplet. The anhydride molecules are then hydrolyzed within the bilayer of the vesicles.[13] After 6 h, the overall OGV mass had increased by a factor of 9. The number and the size distribution (Figure 1B) of the vesicles are significantly changed (note also the presence of concentric intravesicular vesicles, which we will refer to as "inclusion vesicles"): the number of OGVs between 0.5 and 2 μm decreases, whereas new populations of larger vesicles appear. This increase in OGV size could be due to vesicle fusion. In our case, however, we have no evidence that this takes place. Since the mean size of vesicles increases with time, the self-reproduction of OGV has a special connotation, as it does not correspond simply to an increase of the population of a monodisperse species, as in the case of micelles.[14]

Among all the different vesicle transformations observed,[15] here we limit ourselves to describe the major events which lead to an increase of number and/or size of the vesicles. One of these events is the transformation of some of the OGVs into inclusion vesicles (Figure 1B, Figure 2A−C). This transformation occurs in about 20−40% of the OGVs, and it is most likely due to the fact that some of the spontaneously formed vesicles are not unilamellar but rather composed of more than one closely packed bilayer.[16] The hydrolysis of externally added oleic anhydride then occurs at the outermost layer, leading to a swelling of this double layer, resulting eventually in the formation of inclusion vesicles. In some cases the internal vesicle adheres to the outer vesicle shell, from where it is translocated within a few seconds across the bilayer of the outer vesicle (Figure 2D−F), similarly to the process observed by Menger and Gabrielson.[10b] Another event observed is vesicle budding (Figure 2G−I), leading again to an increase of the number of vesicles. In this case, the vesicle reproduction mechanism involves the formation of a new vesicle out of the membrane of an already existing vesicle (Figure 2H).

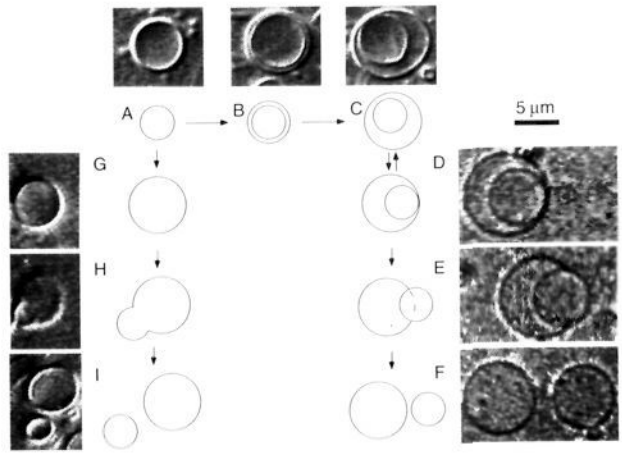

Figure 2. OGV transformations and two self-reproduction processes following intralamellar oleic anhydride hydrolysis. (A−C) Formation of "inclusion vesicles" (picture b was taken 2 h after A; C was taken 8 h after B). (D−F) Vesicle "birthing" (picture E was taken 5 s after D; F was taken 2 s after E). (G−I) Vesicle budding (picture H was taken 10 s after G; I was taken 5 s after H). The scale bar represents 5 μm for all micrographs. Other transformations include, for example, the formation of ellipsoidal and tubular structures.[15]

With respect to literature work which describes transformations of giant vesicles due to the mechanical addition of surfactant to double chain lipids[10b] or due to osmotic or temperature-induced changes,[6,10a] the present work has three salient novel features: (i) we are dealing with a chemical reaction which yields the surfactant *in situ*, (ii) the reaction is catalyzed by the vesicles themselves and (iii) the overall process can be seen as an autopoietic self-reproduction.

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(13) In the case of small vesicles, the binding of anhydride molecules to the vesicles has been shown by infrared spectroscopy.[2b]

(14) Bachmann, P. A.; Luisi, P. L.; Lang, J. *Nature* **1992**, *357*, 57−59.

(15) Results on other shape transformations of vesicles occurring during the anhydride hydrolysis reaction will be published later on.

(16) For about 40−50% of the OGVs, the anhydride hydrolysis leads to a steady increase in size, without formation of an internal vesicle. In this case, the OGVs at the beginning of the reaction were probably unilamellar. Cryoelectron microscopic analysis is in progress in order to determine the lamellarity of spontaneously formed OGVs.