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Rapid Electroformation of Giant Vesicles

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With a small amount of an electrolyte, electroformation of giant vesicles (GVs) proceeded faster than in pure water. In 50 μM neutral phosphate buffer, a GV larger than 50 μm formed in 100 s. The time required for the completion was 5–10 min while electroformation in pure water usually takes 90–120 min. The rapid formation was observed with various electrolytes but only of appropriate concentration (for phosphate buffer, 40–200 μM) and not with a nonelectrolyte such as glucose.

Cell-sized lipid bilayer membrane vesicles (giant vesicles, GVs) are indispensable as a cell membrane model[1,2] in various biophysical/biochemical studies of functional molecules or in construction of a membrane-based microchemical system known as an artificial cell.[3] For GV preparation,[2] electroformation has been frequently used because it yields unilamellar GVs of relatively large size.[4] The procedure is based on enhanced swelling of a thin lipid deposit layer by applied electric voltage (usually sinusoidal AC of low voltage and frequency) in an aqueous medium. The swelling proceeds in a slow gradual manner. Shimanouchi and co-workers recently studied the kinetic aspect of electroformation and reported that the completion of the GV formation takes approximately 90 min under their optimal conditions (1.5 V, 10 Hz).[5]

Concerning the effect of ionic strength of the medium, high concentration of electrolytes is known to deter electroformation.[6] The interference diminishes as the concentration becomes lower, and it is, therefore, assumed that the formation should best proceed in pure water. However, electroformation in the presence of very low concentration of electrolytes has not been investigated explicitly.

In this study, electroformation was examined in aqueous solutions containing a small amount of electrolyte ions (<10 mM). We found significant enhancement of the rate of GV formation under the conditions.

A standard electroformation chamber was constructed with two parallel platinum wires (diameter 0.5 mm, separation 5 mm) as electrodes.[4,6] A methanolic solution (0.50 μL, 1.0 mg lipid/mL) of egg yolk phosphatidylcholine (EggPC; Avanti Polar Lipids, Alabaster, AL) was deposited on the electrode. After drying, the chamber was filled with pure water or an appropriate aqueous solution, and sinusoidal AC voltage was applied between the electrodes from a function generator. The swelling of the lipid was observed on an inverted optical microscope equipped with a digital image enhancement system (Olympus IX-50, Tokyo, Japan). The diameter of large spherical vesicles (>20 μm) was determined and given as a range characteristic to typical formation under the current conditions. Smaller GVs, "mushrooms" or GVs partially hidden by the electrode were excluded from the evaluation.

First, for reference, electroformation in Milli-Q grade ultrapure water was examined. At 4 min after the application of AC voltage (5.0 Vpp (peak-to-peak), 2.0 Hz), hemispherical "domes" or "mushrooms" were seen (Figure 1a). Then, small GVs (approximately 20 μm) began to appear (Figure 1b), and the membranous objects gradually grew to larger GVs (20–50 μm; Figure 1c). The completion of the formation required 90–120 min, which is typical for the standard electroformation procedure.[4,5]

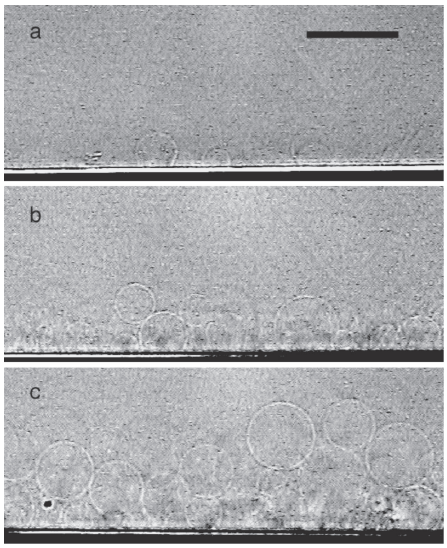

Figure 1. Electroformation of giant vesicles from EggPC under AC voltage (5.0 Vpp, 2.0 Hz) in ultrapure water. (a) 4, (b) 20, and (c) 120 min after the application of the voltage. The electrode appears as the black shadow at the bottom of the frame. The bar indicates 50 μm.

Then, we investigated electroformation in a neutral phosphate buffer solution of low concentration (50 μM, pH 7.0). In this case, membranous domes and mushrooms started to appear immediately after the application of AC voltage and rapidly grew to large GVs (Figure 2a). Some GVs were larger than 50 μm (Figure 2b). Typically, 40 to 100 GVs were found after 5 min, and no further growth or formation of large GVs was observed thereafter (Figures 2b and 2c).

We successfully caught the moment of the rapid formation of a single GV (Figure 3). It seems to be an accelerated version of standard gradual electroformation.[6,7] First, a small semivesicular membranous object appeared (Figure 3a). It rapidly grew by inflation and/or fusion (Figures 3b and 3c) to a larger one. Reshaping (Figures 3d and 3e) yielded a spherical GV (Figure 3f). The whole process occurred in less than 100 s.

At the same time, some of the semivesicular objects formed in the early stage collapsed and disappeared by the end of the formation as also seen in the left parts of the pictures in Figure 3. Probably, those membranous objects could not tolerate

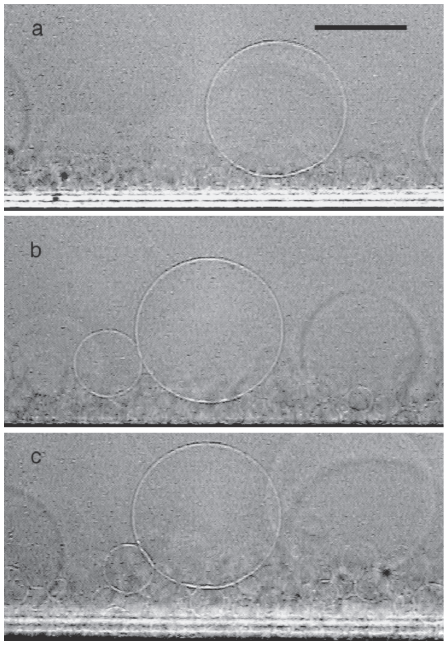

Figure 2. Rapid electroformation in 50 μM phosphate buffer (pH 7.0). (a) 70 s, (b) 7, and (c) 30 min after the application of the voltage (5.0 Vpp, 2.0 Hz). The electrode appears as the black shadow at the bottom. The bar indicates 50 μm.

the stress of the rapid membrane inflation or fusion. Due to the collapse, the rapid formation usually yields less GVs that are larger than 20 μm compared with the conventional electroformation.

A few conditions are crucial for the rapid formation. First, the presence of an electrolyte is essential. Glucose solutions of comparable osmotic strength (62.5–250 μM; pH 6–7) showed no noticeable effect on GV formation, and the electroformation proceeded conventionally. Meanwhile, other electrolytes can induce the rapid formation. For example, in an aqueous sodium chloride solution (100 μM), similar rapid formation of GVs was observed. However, the number of the formed GVs was approximately half, and the size of the vesicles was slightly smaller (20–30 μm) than that obtained in neutral phosphate buffer.

The rapid formation of GVs occurs only in a certain range of the electrolyte concentration. We tested the swelling with neutral phosphate buffer solutions of various concentrations (5 μM to 10 mM), and the rapid formation was observed only in the range between 40 and 200 μM. At the lower concentration (5 or 25 μM), GVs formed as in pure water. Beyond the optimum range, small GVs formed only sparsely at 1.0 mM, and no GVs were seen at 10 mM, confirming the known deterioration of electroformation under relatively high salt concentration.[6]

The rapid swelling of a lipid deposit required the applied electric voltage. Incubation in neutral phosphate buffer (50 μM) without the voltage resulted in no appreciable formation of GVs in 5 min. Upon following application of the voltage (5.0 Vpp, 2.0 Hz), GVs formed rapidly. The voltage is also essential for continuation of the rapid formation. Termination of the applied voltage in the middle of GV growth immediately stopped the inflation at that point.

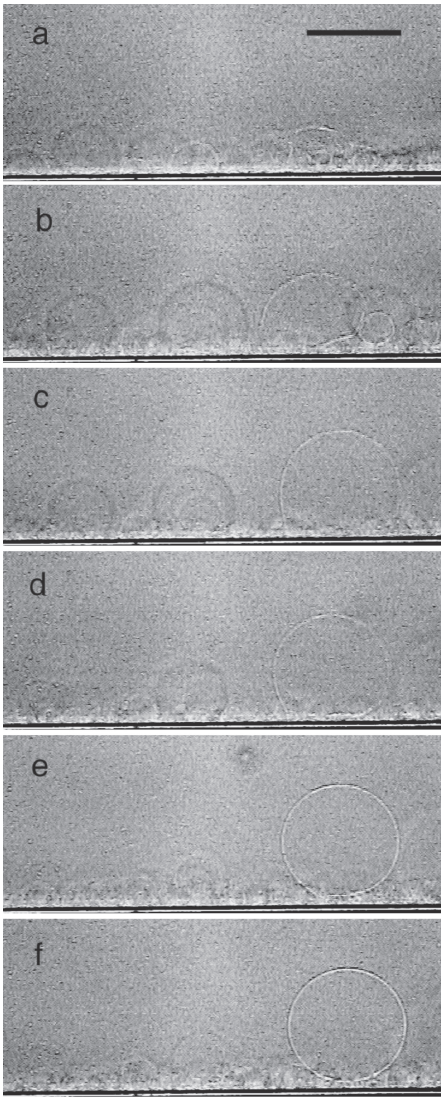

Figure 3. Rapid formation of a single giant vesicle in 50 μM phosphate buffer. The formation started immediately after application of AC voltage (5.0 Vpp, 2.0 Hz). (a) 20, (b) 35, (c) 40, (d) 50, (e) 60, and (f) 95 s. The bar indicates 50 μm.

A swelled lipid deposit that failed to form large GVs in rapid electroformation could no longer yield large GVs by following extended application of the electric voltage to 120 min. Ordinary gradual electroformation of large GVs as seen in Figure 1 was not observed. Instead, the lipid layer formed small vesicles and irregular membranous objects. Electroformation of large GVs seems to require particular lipid organization in the lipid deposit in the early stage of the process. A portion of lipid deposit that satisfies the conditions produces large GVs in rapid formation. One that does not meet the requirement or collapsed during the rapid growth is not able to form large GVs even gradually.

Encapsulation into the aqueous interior of the rapidly formed GV was also examined. The electroformation was performed in a 0.4% (by weight) solution of fluorescein isothiocyanate conjugated dextran (FITC-Dex, approximate average molecular weight 70000; purchased from Sigma-

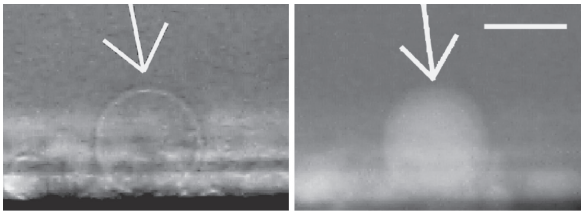

Figure 4. Encapsulation of FITC-Dex in a GV prepared by rapid electroformation. The GV was formed in a 0.4% solution of fluorescein isothiocyanate conjugated dextran (FITC-Dex, approximate average molecular weight 70000) dissolved in 50 μM neutral phosphate buffer. After the formation, the external aqueous phase was gently replaced by pure water. The GV was observed through visible light (left) and fluorescence (right). The bar indicates 20 μm.

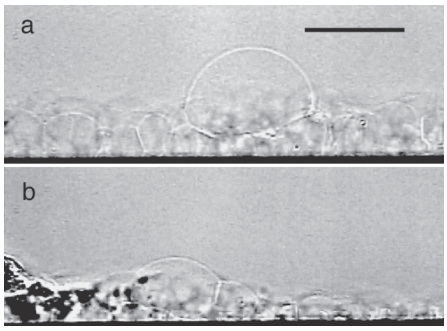

Figure 5. Osmotic shrinkage of GVs. After electroformation, the glucose concentration in the external aqueous phases of the GVs was increased to 1.0 mM. The GVs were formed by ordinary (above) and rapid (below) electroformation. The bar indicates 50 μm.

Aldrich, St. Louis, MO) dissolved in 50 μM neutral phosphate buffer. No effect of the FITC-Dex on the progress of the rapid formation was observed. After the formation, the external aqueous phase was gently replaced by pure water. Inspected with a fluorescence microscope, the GVs thus obtained showed visible emission from the inner aqueous phase of the GVs, indicating encapsulation of the FITC-Dex during the formation (Figure 4).

As an examination of the physicochemical properties of a rapidly formed GV, osmotic shrinkage of the GV was tested and compared with that obtained by conventional electroformation in pure water. After the GV formation, the electric voltage was turned off, and the glucose concentration in the external bulk aqueous phases of the GVs was increased to 1.0 mM. Both the GVs started to shrink and almost collapsed. No significant difference was observed in the shrinkage behavior between the two GVs (Figure 5).

The lipid deposit on the electrode immersed in an aqueous solution is thought to consist of stacking layers of lipid bilayer membranes with many defects.[4,5] Although the mechanism of electroformation or lipid swelling has not been fully elucidated, formation of a GV by the swelling should require separation of lipid bilayer membrane by penetration of water into the intermembrane space.[2] In the present case of the rapid formation, the electrolyte concentration might be too low to expect large osmotic effect or reduction of attractive intermembrane interaction by ions.[8]

As the major driving force of electroformation, electro-osmotic flow (EOF) has been suggested.[4] Conventional EOF decreases as ionic strength of the aqueous medium increases due to compression of the electrical double layer (EDL).[9] The observed deterioration of GV formation at the higher electrolyte concentration is consistent with this prediction. On the other hand, EOF in pure water is less well understood. Fujiwara and co-workers studied EOF in a thin water layer of very low conductivity between two planar glass plates and reported that nonlinear EOF became larger as the conductivity increased.[10] They attributed the deviation from the theory to the low ion density. In the present case, EOF also seems to be suppressed in pure water because of the ion depletion. The increase of the electrolyte concentration remedies the situation and results in the rapid formation.

In their early pioneering study of electroformation, Angelova and her co-worker mentioned that application of DC voltage for 10 s on EggPC deposit resulted in GV formation in distilled water.[11] However, this point has not been further pursued. We are presently working on elucidation of relation between their observation and the present results by investigating the further detail of the rapid formation.

The present study demonstrated that a very small amount of electrolytes could regulate the fate of the lipid layer in electroformation. It is surprising that such a subtle difference in the electrolyte condition could have a significant effect on the progress of electroformation. It also showed that under the optimized conditions, formation of GVs by swelling of lipid membrane layer could proceed quite rapidly. To our best knowledge, the present case is one of the fastest examples of GV formation based on controlled lipid swelling clearly demonstrated to date.

References

1 *Giant Vesicles: Perspectives in Supramolecular Chemistry*, ed. by P. L. Luisi, P. Walde, John Wiley & Sons, Chichester, **1999**.
2 P. Walde, K. Cosentino, H. Engel, P. Stano, *ChemBioChem* **2010**, *11*, 848.
3 P. L. Luisi, F. Ferri, P. Stano, *Naturwissenschaften* **2006**, *93*, 1.
4 M. I. Angelova, in *Giant Vesicles: Perspectives in Supramolecular Chemistry*, ed. by P. L. Luisi, P. Walde, John Wiley & Sons, Chichester, **1999**, p. 27.
5 T. Shimanouchi, H. Umakoshi, R. Kuboi, *Langmuir* **2009**, *25*, 4835.
6 P. Bucher, A. Fischer, P. L. Luisi, T. Oberholzer, P. Walde, *Langmuir* **1998**, *14*, 2712.
7 M. I. Angelova, S. Soléau, P. Méléard, F. Faucon, P. Bothorel, *Prog. Colloid Polym. Sci.* **1992**, *89*, 127.
8 H. I. Petrache, S. Tristram-Nagle, D. Harries, N. Kučerka, J. F. Nagle, V. A. Parsegian, *J. Lipid Res.* **2006**, *47*, 302.
9 *Fundamentals of Interface and Colloid Science: Solid-Liquid Interfaces*, ed. by J. Lyklema, Academic Press, London, **1995**, Vol. II.
10 T. Fujiwara, O. Kitoh, T. Tsuda, *Chromatography* **2002**, *23*, 25.
11 M. Angelova, D. S. Dimitrov, *Prog. Colloid Polym. Sci.* **1988**, *76*, 59.

